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# BIOCHEMICAL RESEARCH

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ALBERT P. MATHEWS

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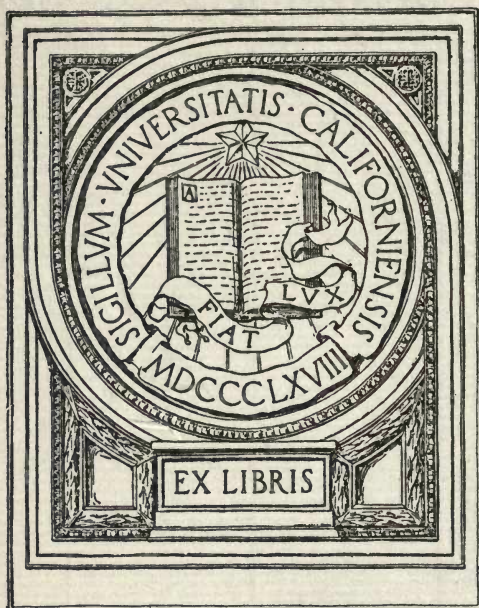


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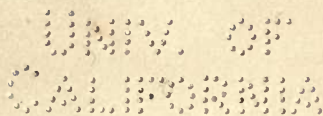


Waldemar Kersh.



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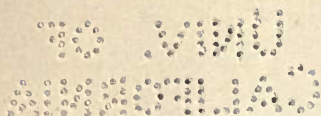
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DEDICATED TO THE MEMORY  
OF  
WALDEMAR KOCH  
(April 8, 1875—February 1, 1911)

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## WALDEMAR KOCH

Herman Koch, a mining engineer of international reputation, lived in Clausthal, Hannover, Germany. His father, his grandfather and his grandfather's father had been mining engineers before him. One of his nine sons was Robert Koch, the great bacteriologist; another was Hugo Koch, also a mining engineer; another, Arnold Koch, came to this country in 1867 with letters of introduction from Alfred Nobel, who was a friend of Herman Koch. Arnold Koch settled in St. Louis, where his only son, Waldemar, was born April 8, 1875.

The first part of Dr. Koch's college life was spent in Washington University, St. Louis, but his last year he spent in Harvard, from which he received his undergraduate degree, and two years later, in 1900, the degree of Ph.D. in organic chemistry. He was then for one year assistant in physiology in the Harvard Medical School with Professor Porter. He began at that time the study of the chemistry of the nervous system, which he continued until his death. He came to the University of Chicago as an associate in physiological chemistry in 1901, and with a short interregnum spent in teaching pharmacology and physiological chemistry in the University of Missouri, he remained in the University of Chicago continuously thereafter, where at the time of his death he was associate professor of pharmacology. He was for a time with Schmiedeberg in Strassburg; and during his vacations he worked for several years, part of the time under a grant from the Rockefeller Institute, in the laboratory of Dr. Mott in the Claybury Asylum for the Insane, near London; afterwards for one season he was on the staff of the new hospital for the insane at Long Grove, near London; and for the past year he had been connected, also, with the Wistar Institute of Anatomy in Philadelphia. In these various institutions he had unusual opportunities, which he utilized to the utmost, for the study of pathological and normal nervous material.

Dr. Koch's work on the chemistry of the nervous system is

known to all physiological chemists. The chemistry of the brain is a very difficult field and the separation of the lipoid substances is still hardly possible. He spent the greater part of these years in devising accurate methods of quantitative analysis. These methods had been so perfected that he had secured more complete and more accurate quantitative analyses of nervous tissue than any hitherto made. By means of these methods he was attacking the problem of the differentiation of the brain during growth; the distribution of various substances in different parts of the brain; variations in composition during disease; and the differences between the brains of different animals. He was also engaged in separating carefully the various lipoids, such as kephalin and lecithin, and in examining their composition. He showed the very important fact that kephalin exists as a potassium salt, whereas lecithin has more of an affinity for sodium. His method of purification of the lipoids by precipitation with chloroform and hydrochloric acid was extremely useful. Among his other important contributions must be mentioned his work on the behavior of lecithin and kephalin emulsions towards various salts, anesthetics and drugs, work which showed one way in which these substances might influence irritability. He discovered, also, that the brains of persons having the very obscure insanity, dementia præcox, contained less of a certain sulfur fraction than usual, and in his further examination of the sulfur distribution in the brain he isolated a lipoid-sulfur compound of very interesting nature. He had prepared a considerable quantity of this compound and he was engaged in studying its nature at the time of his death.

Dr. Koch's interest from the first had been in the problem of the action of drugs on the nervous system. He taught pharmacology almost from the time of his graduation. He fitted himself for his duties as a teacher by taking the regular medical courses in pathology, anatomy, embryology and many of the clinical courses, as well as by studying with Schmiedeberg in Germany. He thus had a very unusually broad training and was able to look at his subject from all sides, the chemical, the biological, and the clinical. There are very few men in pharmacology to-day, who possess his extensive knowledge and his sane, scientific and broad point of view.



The work he was doing on the nervous system he regarded as fundamental work in pharmacology, necessary before any real science of pharmacology could be constructed. He had just reached a point when the direct application of his methods to the solution of the problem of how drugs combine with nerve cells could be begun. To die at such a time was particularly cruel. Had he lived a few years longer his recognition as one of the leading pharmacologists of the world would undoubtedly have been assured. His work, like that of his uncle, Robert Koch, was very thorough and exact, and he proceeded in logical order to overcome one difficulty after another.

Dr. Koch's personality won him many friends. He was a true, loyal and courageous friend, entirely honest and of sane judgment; he avoided making enemies, as far as possible. He was fond of out of doors, and loved the hills, rivers and fields, and tramping in the dunes near Chicago was his main recreation. He had a keen appreciation of what was fine in music and in art. His was an open, frank, kindly nature, considerate of others and slow to anger.

His death by pneumonia, February 1st, at the early age of thirty-six, was an irreparable loss to his friends, to his University and to science.

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STUDIES ON THE PHYSICAL PROPERTIES OF  
PROTOPLASM



## STUDIES ON THE PHYSICAL PROPERTIES OF PROTOPLASM

### I. THE PHYSICAL PROPERTIES OF THE PROTOPLASM OF CERTAIN ANIMAL AND PLANT CELLS

BY G. L. KITE

[From the Hull Laboratories of Biochemistry and Pharmacology, University of Chicago]

#### INTRODUCTION

ALTHOUGH the living substance of animal and plant cells was correctly interpreted by Dujardin and von Mohl in the second quarter of the nineteenth century, almost nothing is definitely known of the physical state of protoplasm. Properties described by such adjectives as glutinous, slimy and hyaline were recognized by the early microscopists, who were forced to study living cells and tissues.

During the last fifty years an extensive literature has grown up on the subject of the structure of protoplasm. For the purposes of this paper, these investigations may be divided into two groups. The first group comprises those studies on the structure of protoplasm, made with the aid of fixatives. A large part of our knowledge of the morphology of the cells and tissues of animals and plants is the direct result of the development of fixing methods.

The errors involved in the attempts to determine the true molar structure of protoplasm by the use of fixing reagents have been pointed out particularly by Flemming,<sup>1</sup> Berthold,<sup>2</sup> Schwarz,<sup>3</sup> Fischer,<sup>4</sup> and Hardy.<sup>5</sup> In this connection, it will suffice to state that Hardy's conclusion that fixing reagents always cause structural changes in pro-

<sup>1</sup> FLEMMING: Zellsubstanz, Kern und Zelltheilung, 1882, Leipzig.

<sup>2</sup> BERTHOLD: Studien über Protoplasmamechanik, 1886, Leipzig.

<sup>3</sup> SCHWARZ: Cohn's Beiträge zur Biologie der Pflanzen, 1887, v, p. 1.

<sup>4</sup> FISCHER: Archiv für Entwicklungsmechanik, 1901, xiii, p. 1.

<sup>5</sup> HARDY: Journal of physiology, 1899, xxiv, p. 158.

toplasm that are frequently very different from the normal living substance, has never been refuted. Hence, at least, it does not seem that more than an approximation of the actual structure of protoplasm can be attained by the use of fixatives. Besides, this method is worthless as a means of investigating the physics of protoplasm.

The papers that fall in the second group deal with the study of living cells. Strassburger,<sup>6</sup> Wilson,<sup>7</sup> Foot and Strobell,<sup>8</sup> Lundegardh<sup>9</sup> and others have shown that many of the structural elements of the mitotic figure can be seen in living animal and plant cells. Numerous investigators have pointed out the presence of granules, vacuoles, and fibrils in various types of unfixed cells; but for the most part, the studies on living cells have been made for the purpose of decreasing the error due to the use of fixing reagents.

All such investigations are open to several sources of error. Hardy<sup>10</sup> writes that the process of dying produces structural changes in the cell substance, since coagulation appears to occur in all dying cells. Many cells are certainly quickly asphyxiated when mounted for microscopical examination in the usual manner. I have been able to overcome, largely, this source of error, by the use of an open-end moist chamber, that does not appear to interfere with normal respiration.

A second source of error is due to the nature of the optical principles involved in microscopical vision. Many years since Abbé<sup>11</sup> demonstrated that the optical image is a diffraction pattern produced by the object and that under certain conditions the image may be quite different from the object. More recently, Porter,<sup>12</sup> experimenting under ordinary working conditions, has described a number of interesting examples of this sort. Porter<sup>12</sup> says, "Images were formed which were utterly false in their smaller details, and other images were profoundly modified by the presence of structure lying

<sup>6</sup> STRASSBURGER: *Zellbildung und Zelltheilung*, Jena, 1880, iii. Auflage.

<sup>7</sup> WILSON: *Journal of morphology*, 1899, xv, Suppl.

<sup>8</sup> FOOT and STROBELL: *American journal of anatomy*, iv, p. 199.

<sup>9</sup> LUNDEGARDH: *Jahrbücher für wissenschaftliche Botanik*, li, p. 236.

<sup>10</sup> HARDY: *Journal of physiology*, 1899, xxiv, p. 158.

<sup>11</sup> ABBÉ: *Archiv für mikroskopische Anatomie*, 1874, ix, p. 413; *Gesammelte Abhandlungen*, 1904, i, p. 45.

<sup>12</sup> PORTER: *Philosophical magazine*, 1906, xi, p. 154.

entirely beyond the focal plane." Such facts should serve to make it evident that one can easily fall into error in interpreting the optical image of a living cell. Porter<sup>12</sup> recommends "a working knowledge of the phenomena and laws of diffraction," as a safeguard against this form of error.

The third and by far the most important source of error is due to the peculiar and little known optical properties of living matter. The phenomena of reflection, refraction, absorption, dispersion, interference, diffraction<sup>13</sup> and a scattering action on light<sup>14</sup> are all exhibited by this substance, with the result that a correct interpretation of the image of a living cell is frequently impossible. Furthermore, many cells are so opaque and turbid that the interior is not visible. Cloudiness or turbidity is almost a universal property of protoplasm and appears to be due chiefly to dispersion, refraction, diffraction, and the scattering action on light of the colloidal particles which may be considered as the real structural units of all protoplasm.

Globules, granules and cell walls frequently show diffraction halos that are difficult to interpret in undissected cells.

The aim of this investigation is to determine the physical state and the molar structure of protoplasm. The methods are radically different from those heretofore used, and are believed to be adequate for this purpose. Dissection and vital staining are used to determine the truthfulness of the optical image and the actual structure of cells. Unfortunately, the amount of the error involved in the employment of these methods depends entirely on the skill of the experimenter; but it is believed that the error becomes quite small with complete mastery of the methods.

The structural changes that cells may undergo during the time

<sup>13</sup> Excellent expositions of the principles of physical optics are given by: WOOD, R. W: 1911, *Physical Optics*; DRUDE, P.: 1912, *Lehrbuch der Optik*; PRESTON: 1901, *Theory of Light*.

<sup>14</sup> Lord Rayleigh (*Philosophical Magazine*, xli, p. 107) has pointed out that reflection and refraction have no application unless the surface of the disturbing body is larger than many square wave-lengths. The turbidity of protoplasmic sols, then, is due entirely to the scattering action on light of the minute aggregates of the disperse phase, while reflection, refraction, diffraction, dispersion and a scattering action on light are all seemingly involved in the production of turbidity by gels.



required for their dissection is a possible source of error that may appear, at first sight, to be very difficult to control. Certainly many biologists hold the view that cells rapidly undergo important morphological changes following mechanical injury. With few exceptions it has not been found difficult to follow the structural changes that occur in cells that are being dissected; but the really remarkable fact is the marked slowness of such death changes as granulation, fragmentation and general coagulation, following mechanical injury.

It seems best to limit this introductory paper to a description of selected types of widely different cells and in future publications to treat systematically, selected types of the principal phyla of animals and the chief groups of plants.

The special literature bearing on this investigation will be discussed in subsequent papers.

A review of such well-known theories as those of Bütschli, Flemming and Altmann lies outside the province of this paper.

#### METHODS AND MATERIAL

The development of a really adequate method for the dissection of living cells, under the highest powers of the microscope, has made possible this study. The principles of this method are simple. The dissecting instrument is a glass needle that may measure less than one micron in diameter and is drawn on the end of a piece of special Jena glass tubing about 200 mm. long and 4 mm. in diameter. The needle is held in a three-movement Barber pipette holder. The cell chosen for dissection is mounted in a hanging drop in an open-end moist chamber and held in place by water-glass surface tension, which can be varied at will.

Both diffuse sunlight and artificial light are used as sources of illumination. For the latter a Nernst Glower has been found satisfactory, but all the light waves outside of 450 and 670  $\mu\mu$  are cut out by the use of appropriate ray screens. The same means is used to remove enough of the orange and red rays to make the transmitted light perfectly white. Such light is composed of the waves that are least injurious to living cells. A special condenser<sup>15</sup> of a focal dis-

<sup>15</sup> The condenser was made by E. Leitz & Co., Wetzlar.

stance of about 20 mm., a 2 mm. apochromatic objective, compensating oculars, and a number of vital stains, are necessary additions.

An open-end moist chamber 25 x 60 x 15 mm. has been found satisfactory. The bottom is separated into three compartments by very small glass rods and water is placed in the end compartments. If water be put in the middle compartment it may decrease the efficiency of the condenser. The chamber is held in a mechanical stage and most of the dissections are made by quick movements of the chamber and therefore of the cell being dissected, the needle remaining fixed.

The use of acetylene which can be burned in a glass micro-burner has greatly simplified the making of extremely fine needles. An acetylene flame that is so small that it is invisible in a well-lighted room can be kept alive.

The more important of the vital stains used include methylene blue, new methylene blue N (Cassella Color Co.), new methylene blue GG (Cassella Color Co.), new methylene blue R (Cassella Color Co.), janus green (Metz & Co.), pyronin (Grübler), vusuvín (Grübler), toluidin blue (Grübler), neutral red (Grübler).

The chief structural components of a cell can usually be quickly brought out by using a large enough number of vital stains, thus effecting a great economy of time, when the dissection of the unstained cell is made.

Barber's<sup>16</sup> isolation and intracellular injection methods, variously modified, are frequently employed to supplement and control the data obtained by dissection.

**Nomenclature Employed.** — The current nomenclature of descriptive physics, physical optics and colloidal chemistry will be employed. Such physical properties as solidity, tenacity, elasticity, hardness and viscosity have been determined for the cells, so far studied. In general, the term viscosity will be used to designate the degree of rigidity of protoplasmic structures, but such a structure as a vitelline membrane may be comparatively soft and yet have what must be considered as a high internal friction or viscosity. Elasticity is determined by transfixing a selected piece of a cell and stretching it and observing the power of resumption of the original form. Dis-

<sup>16</sup> BARBER: University of Kansas Science Bulletin, 1907, iv, p. 3; Journal of infectious diseases, 1911, viii, p. 248; *ibid.*, 1911, ix, p. 117.



section is the method employed for determining such properties as solidity, hardness and tenacity or cohesiveness. All physical properties that have been enumerated are relative and it is hoped at a later time to increase the accuracy of description by the selection of arbitrary standards. The usage of the terms employed in this paper is based on the dissection of many widely different types of animal and plant cells.

Living matter occupies an intermediate position between true solids and true liquids and has many of the properties of both as well as properties peculiar to itself. It belongs to the class of colloids known as emulsoids and exists in either a gel (hydrogel) or a sol (hydrosol) state.<sup>17</sup> The term gel will be used to designate the amorphous semi-solid state and sol the apparently homogeneous liquid state, of living substance. Protoplasmic sols usually appear as hazy homogeneous liquids on account of the very minute size of the protein aggregates that compose the solid phase. On the other hand protoplasmic gels are characterized by the large size of the particles of the solid phase which set to form the gel. Hence, living gels may exhibit either a homogeneous or heterogeneous molar structure.

It should now be clear that the term homogeneous is used in a relative sense to describe the optical image and refers only to the molar structure that can be brought out by the usual microscopical powers and further that heterogeneity is the universal distinguishing characteristic of colloidal sols and gels. In this connection it may be noted that Pauli<sup>18</sup> states that the "unfixed" gel of gelatine is not structured in the sense of being composed of threads, networks, granules and vacuoles; it has the molar structure of a one-phase system, which is precisely what is meant by the term homogeneous as used in this paper; the molecular structure is unknown. The present unsettled state of the problem of phase relations of colloidal

<sup>17</sup> For discussion of the classification of colloids see: NOYES, A: 1905, *Journal of the American Chemical Society*, 1905, xxvii, p. 85; OSTWALD, WO.: 1907, *Zeitschrift für Chemie und Industrie der Kolloide*, 1907, i, p. 291; PERRIN, J.: 1905, *Journal de la chimie physique*, iii, p. 50; FREUNDLICH and NEUMANN, 1908, *Kolloid Zeitschrift* iii, p. 80; VON WEIMARN, P. P.: *ibid.*, 1908, iii, p. 26.

<sup>18</sup> PAULI: *Der Kolloidale Zustand und die Vorgänge in der lebendigen Substanz*, Braunschweig, 1902.



solutions has been ably discussed in a recent paper by Hardy.<sup>19</sup> It is usual to regard colloidal systems as consisting of two phases, a solid and a liquid, which have been termed by Wo. Ostwald<sup>20</sup> the disperse phase and the dispersion medium, respectively.

For convenience of description arbitrary meanings will be given the terms microsome and globule; the former will be restricted to minute dense masses of gel, the latter to suspensions in protoplasm that show many of the physical properties of oil droplets and besides are usually free of protoplasm when dissected out of a cell. Most of the suspensions so far found in cells fall into one or the other of these groups, but intermediate forms have been observed.

#### THE EGG OF ASTERIAS

The egg of *Asterias* is surrounded by a mass of either transparent or translucent jelly which is soft and somewhat elastic and glutinous; but it can be cut and torn to pieces and removed from the egg with little difficulty. Thirty-four and six-tenths microns is the average thickness of this jelly. This structure has a low viscosity for a gel and is therefore extremely dilute. On many eggs, the jelly has become turbid and undergone a change in refractive power and as a result is visible in the usual microscopical examination. The inner surface of the jelly envelope is closely applied to the outer surface of the vitelline membrane which is invisible except in eggs that have matured. The vitelline membrane of the immature starfish egg is a transparent and invisible solid of about two microns in thickness. The physical properties of this structure are very definite since it exhibits extraordinarily high viscosity, elasticity and tenacity. A small piece can be drawn out into a mere thread and when freed the thread contracts to a more or less rounded mass. During maturation the vitelline membrane swells to two and three times its original thickness, undergoes a change in refractive index, and becomes quite cloudy and hence visible. In this state it is softer, more glutinous and less rigid. The inner surface of this

<sup>19</sup> HARDY: Proceedings of the Royal Society, Series A, 1912, lxxxvi, p. 601.

<sup>20</sup> OSTWALD: Zeitschrift für chemie und Industrie der Kolloide, 1907, i, p. 291.

membrane is tightly glued to the surface of the cytoplasm, from which it can be dissected only with considerable difficulty.

The misleading optical phenomena that are involved in a study of the cytoplasm are of great interest.

It is usual for cytologists to consider the echinoderm egg a classical example of the alveolar structure of protoplasm. No one can question the fact that beautiful round spaces with hazy, protoplasmic walls in which are embedded minute granules, can be seen in such eggs. Bütschli supposed these spaces to be filled with a watery fluid.

What is the true structure of the cytoplasm of the egg of *Asterias*? Careful dissections give a clear-cut answer to this question.

The cytoplasm is a quiet translucent gel of comparatively high viscosity; it can be drawn out into large strands, but is not cohesive and elastic enough to form small threads. It can be cut into small pieces with comparative ease. Fragments usually become spherical, though in some cases water is slowly taken up and the mass changes into the sol state. Minute granules measuring little more than one micron are scattered plentifully throughout the cytoplasmic gel. It has been found impossible to free these structures completely from the gel in which they are embedded. They are optically more dense and have a different refractive index from the surrounding living substance. A part of the total mass of cytoplasm is composed of what appears to be alveoli or spaces; but a careful dissection of such an alveolus reveals the presence of a globule that has many of the optical properties of an oil drop. Such a globule, freed from cytoplasm, does not dissolve in sea water and in a light of low intensity exhibits the usual diffraction halo. The invisibility of liquid droplets of rather high viscosity when embedded in the cytoplasm might at first sight appear difficult to explain. This invisibility is due to the fact that the refractive index and dispersive power of the globules is very near that of sea water; also, the optical density of the cytoplasm is evidently higher than that of the globule. No diffraction rings could be seen surrounding the globules when they were imbedded in cytoplasm. Centrifugal force dislodges the globules, proving them to be merely suspended in a living gel. The minute granules respond much less readily to centrifugal force. Besides they show optical properties — their index of refraction is certainly higher than that of the surrounding gel — that ally them to highly concentrated particles

of the cytoplasmic gel. Yet it seems likely that all such structures as granules and globules must be considered as having separated out of the disperse phase and to be therefore of the nature of suspensions. The living cytoplasm, then, is an apparently homogeneous and very viscous gel in which microsomes and globules are suspended.

If the nucleus of the immature starfish egg be dissected out in sea water it undergoes no appreciable change. Dissection of the highly-translucent nuclear membrane shows this structure to be a very tough viscous solid, and, in fact, closely allied physically to the vitelline membrane and not at all the delicate structure of the conventional descriptions. With the exception of the nucleolus, the nuclear substance is all in the sol state. The nuclear sol is apparently a homogeneous liquid. The nucleolus is a small mass of quite rigid and cohesive granular gel that is suspended in the nuclear sol.

The polar body is a granular, elastic and highly viscous gel.

In order to make it possible to observe the structural components of the starfish egg and of the eggs of other common marine invertebrates, without having to use my tedious methods, vital staining was resorted to. The jelly envelope can be stained a beautiful light blue with dimethyl-safranin-azo-dimethyl-anilin; the vitelline membrane a very dark blue with isamin blue; the globules or droplets from yellow to orange with vusuvine; and the extremely small granules a slate blue with diethyl-safranin-azo-dimethyl-anilin.

The dead or dying asterias egg shows remarkable morphological changes. The whole egg becomes almost opaque. The cytoplasm separates into a large number of more or less rounded masses which still adhere to each other. Such masses vary greatly in size, some being as small as five microns in diameter. If the formation of such small masses be observed, one is easily misled into believing that fusion of the globules is occurring. Dissection of such a mass frees the original globules. The dead gel does not stick to a glass needle and can no longer be drawn out into strands; it has lost much of its viscosity and cohesiveness. The nuclear fluid has set and the resulting gel is more voluminous than was the nuclear fluid in the living egg. The nuclear membrane shows little change in its physical properties, while the nuclear gel is elastic and quite viscous and granular. The physical properties of the dead nuclear gel are very similar to those



exhibited by the living cytoplasm. Small fragments of the dead nuclear gel do not go into solution when dissected out in sea water.

#### AMEBA PROTEUS

Small pieces of ectoplasm of proteus can be cut off in distilled water and show no change. This living substance has a moderately high viscosity and cohesiveness; it does not stick to glass needles very readily and little difficulty is experienced in cutting it into pieces as small as the limit of microscopical visibility. Pieces of all sizes appear perfectly homogeneous. The cloudiness of the ectoplasmic gel is a well-known property. The inner three or four microns of the hyaline ectoplasm and particularly the interior of the outer end of small pseudopods, contain varying numbers of minute granules and globules that may measure as much as four or five microns. If these granules and globules are dissected out they do not go into solution. The globules show confusing diffraction rings; but, both globules and granules can be brought out by light staining with diethyl-safranin-azo-dimethyl-anilin. The endoplasm contains a large contractile vacuole in which the presence of protein has not been demonstrated, as yet, and numerous food vacuoles which contain either liquid or liquid and food masses. The same kind of granules and globules are found in the endoplasm as are found in the ectoplasm and the number of these structures varies in different animals. The substance forming the walls of the vacuoles is of much higher viscosity and cohesiveness. The living endoplasmic substance is a very dilute and apparently homogeneous gel that possesses a remarkable affinity for water. The ectoplasm of ameba then is a quite concentrated gel while the interior is quite dilute and is continuously changing its water-holding power in different regions. New methylene blue R and trypan blue are of great value in bringing out the globules, granules and vacuoles.

The nuclear membrane is an extremely thin and moderately tough solid substance. It shows some elasticity and is quite viscous.

The whole of the nuclear substance is a highly rigid and granular gel, the minutest pieces of which show no appreciable change when dissected out in distilled water. A slight elasticity and a definite

glutinicities are exhibited by this substance. There are variations in concentration of the nuclear gel that produce a characteristic but misleading optical image. The nucleus appears to contain an irregular network with granules imbedded in it. The interstices of the network are very small luminous spots which have been misinterpreted to be vacuoles. Many dissections have shown that the granules are very concentrated masses of gel; the network irregularly disposed masses of a diluter gel; and the interstices or light spots the most dilute gel in the nucleus. The so-called network is a part of the nuclear gel that forms a concentration gradient; the interstices and granules may be considered constants connected by the grading network. It should be clearly understood that the network is not made up of definite threads of fibres but of irregular masses of hydrogel that are very dense immediately surrounding the granules, from which they grade into the dilute gel of the interstices. No free liquid was found in the nuclear substance.

When the granules are in focus they appear gray and cloudy or opalescent; when out of focus as dark spots. They measure from less than one to about two microns in diameter.

It seems that a part of the luminosity of the interstices of the network is due to diffraction and not simply to slight absorption of light by this portion of the nuclear substance.

The structural details of the nucleus can be brought out with considerable vividness by staining with janus green (diethyl-safran—in azo-dimethyl-anilin).

Slight cuts in the surface of proteus quickly close. Extensive cuts frequently cause an ameba to explode—in as short a time as two seconds nothing but the nucleus may remain. If the contractile vacuole be cut and its liquid content caused to mix with the cytoplasm the Ameba is immediately destroyed with explosive violence. A relatively large dose of distilled water and even  $\frac{1}{2}$  to 1 molar cane sugar solution or one molar sodium chloride or potassium nitrate give a like result. It is not usually possible to produce more than a temporary vacuole with two molar cane sugar; a large dose of sugar of this concentration usually causes the appearance of granules, globules, fibrils and a hyaline appearance in any portion of the endoplasm into which the injection is made. The doses that were injected varied from about 270 cubic microns to 30,000 cubic microns.

A large number of indicators have been injected into the interior of *proteus* with the idea of determining a possible relation between an excess of  $H^+$  or  $OH^-$  ions and the extraordinary water-holding-power of the endoplasm. Azolitmin, sodium alizarin sulphonate, tropeolin 000 No. 1, methyl orange and congo red, dissolved in from  $\frac{1}{2}$  to  $\frac{3}{4}$  molar cane sugar have been so far employed. A neutral to slightly alkaline reaction is shown by all the indicators. It seems probable then that the concomitant variation in water-holding-power of different regions of the cytoplasm is the mechanism by which *Ameba proteus* moves and is associated with an excess of  $OH^-$  ions.

A number of operations were performed on the ectoplasm of *Ameba proteus* for the purpose of determining the relation between movement and surface tension changes. The results of shallow and deep cuts in the ectoplasm have already been given. The outer 5 to 7 microns of the pseudopods were cut away in some animals, and in others small doses of distilled water were injected into the ectoplasm. The removal of the outer end of a pseudopod was usually followed by rapid closure of the incision. The injection of distilled water into the ectoplasm had no noticeable effect on the formation of pseudopods. By means of such operations the rigid ectoplasm was either removed, for a short time, from a given area of the surface or at least greatly weakened; yet, no tendency to the formation of pseudopods was ever observed, in such weakened surface areas. These facts seem to justify the conclusion that surface-tension changes play a negligible rôle in the movement of *Ameba proteus*. Furthermore, it may be recalled, that the outer surface of *Ameba proteus* is a semi-rigid solid of from 5 to 12 or more microns in thickness, and it has still to be shown, that the changes, in the tension of the surface film, that are commonly assumed to occur, can appreciably affect the underlying semi-rigid ectoplasm.

The nutrient solution in which the amebae were grown was slightly alkaline in reaction.

*Proteus* usually recovers from the large doses of neutral salts and sugar in much less than an hour, almost certainly by throwing them off.



## PARAMECIUM

The living substance of Paramecium is a soft, elastic and somewhat glutinous gel which can be drawn out into strands. It is filled with a large number of vacuoles of various sizes the walls of which are more dense than the surrounding gel. The surface layer is more viscous and cohesive than the interior. Small cuts usually close quickly, extensive deep cuts are either followed by a loss of cytoplasm or a rapid change of the whole cytoplasm into the sol state with almost explosive violence. If the fluid in the contractile vacuole be caused to mix with the cytoplasm a rapid change of this substance into the sol state results. Suspended in the living and apparently homogeneous and rather dilute gel are varying numbers of extremely small granules and small globules. Many of the granules are recently ingested bacteria. Neither the granules nor globules go into solution when dissected free from the cytoplasm. The food masses are granular gels of rather high viscosity.

The optical properties of the meganucleus render its study extremely tedious. It is almost transparent and invisible. Therefore its refractive index and its dispersion are very close to those of water. Dissection has proved the meganucleus to be a gel of higher viscosity than the cytoplasm and to be slightly glutinous and elastic. The meganuclear gel has areas, more dense than the surrounding substance, that may be considered granules.

A complete study of the micronucleus has not been made.

## NECTURUS

**The Striped Muscle Cell.** — The living substance of the striped muscle cell of Necturus is the most viscous, elastic and cohesive of the living gels we have so far considered. The muscle substance sticks to a glass needle and can be drawn out into extraordinarily long threads which when released almost regain their previous shape. The absorptive power and turbidity of this substance are comparatively high.

When the whole or a piece of a muscle cell is stretched the striations become faint or disappear — only to reappear when the tension

is removed. Beautiful but misleading diffraction phenomena are to be observed when a piece of muscle cell is stretched. If the point of a very minute needle be pushed into a muscle cell, it can be moved in one direction about as easily as another.

The optical image of striped muscle is very misleading. Dissections have shown that the dark bands seen in living muscle are produced by concentrated areas of muscle substance which absorb enough transmitted light of low intensity to appear as dark bands in the optical image. I have been unable to dissect out definite fibrils. The substance lying between the concentrated regions and appearing as light bands is a highly viscous, elastic gel and has no physical properties that serve to distinguish it from the surrounding sarco-plasmic gel. By cutting the dark band to pieces, small masses of highly concentrated muscle substance, frequently less than one micron in diameter, are partially freed from the dilute enveloping gel and in light of low intensity show well-defined diffraction halos. The appearance of dark bands in the optical image, then, is produced by absorption of light waves by the concentrated muscle substance; the light bands, by the low absorptive power of the diluter intermediate gel, and the diffraction of the light waves by the edges of the concentrated substance. Striking changes in the optical image that are well known can be produced by increasing the intensity of illumination. The dark band becomes cloudy and more or less opalescent and the light band may show an intersecting dark line or well-defined diffraction fringes just outside the geometrical shadow of the concentrated substance. Hence, absorption, diffraction, refraction and dispersion are involved in the formation of the optical image of striped muscle and the former two particularly when the illumination is of a relatively high intensity.

The nuclear substance is a gel that is for the most part comparatively dilute but contains more concentrated areas in the form of granules and an imperfect network. The appearance of a network in the optical image is due not to definite fibrils but to more concentrated parts of the gel that grade into the diluter nuclear substance.

On the outer surface of the muscle cell is found a highly translucent membrane, the sarcolemma, which is extremely elastic and measures about one micron in thickness. It is stuck to the whole outer surface of the muscle cell and is viscous and cohesive enough

to offer an appreciable resistance to a glass needle a micron or less in diameter. The disagreement among investigators concerning the presence of a sarcolemma is due to the fact that it is transparent and that its refractive and dispersive powers are so nearly the same as those of water. Instead of being the delicate structure of the conventional descriptions, the sarcolemma of the striped muscle cell of *Necturus* exhibits physical properties that are very similar to those of the vitelline membrane of an echinoderm egg.

If a concentrated solution of isamin blue made by boiling in distilled water or .8 per cent sodium chloride be added to freshly teased muscle cells, blue staining of the sarcolemma occurs in ten to fifteen minutes.

**An Epidermal Cell.**—The epidermal cells are embedded in an intercellular gel of extremely high viscosity and considerable elasticity. The substance is tough but softer than many nuclear membranes and shows a relatively high absorptive power. It is also quite turbid. A few globules and granules, varying in size from about one to four microns, that can be easily stained with diethyl-safranin-azo-dimethyl-anilin are to be seen scattered through the intercellular gel.

The whole cell substance is a gel of even higher rigidity than the muscle substance of the same animal. Small pieces cut out of the nucleus or cytoplasm, in distilled water or .8 per cent sodium chloride, show no appreciable change.

The cytoplasm exhibits a high absorptive power and a definite elasticity. Very small granules that seem to be denser cytoplasmic areas are to be seen scattered throughout the turbid cytoplasm. Many cells show radially arranged fibrils, in the outer part of the cytoplasm, which can be partially freed from the surrounding gel by dissection. Such a fibril is physically and optically more dense than the remainder of the cytoplasm.

The nuclear membrane is thin, clear, and quite cohesive and elastic, and has a different index of refraction from the cytoplasm and nucleus.

The nuclear gel is of a higher viscosity than the cytoplasm. The appearance of a network in the optical image of the nucleus is due to concentrated areas in the form of granules and imperfect threads which are not sharply separated from, but grade into, the surrounding diluter



gel. The whole nuclear substance is quite glutinous. No trace of free liquid could be found in the nucleus.

#### SPIROGYRA

The cellulose wall of *Spirogyra* is enormously cohesive; it is cut or punctured with extremely fine Jena glass needles with considerable difficulty. The outer surface is covered by an almost invisible soft gel, that frequently measures five or more microns in thickness and can be stained red with sodium alizarin sulphonate in a neutral or slightly alkaline solution. A layer of dilute granular gel covers the inner surface of the cellulose wall and is connected by a number of strands of an elastic gel to a central mass of living substance, in which a small nucleus is imbedded. The central mass of gel contains a few granules and is of a higher viscosity and cohesiveness than the surface cytoplasm. This mass also has a higher refractive index and higher absorptive power than the surface cytoplasm. The anchoring strands of gel decrease in viscosity from within outwards. Much of the surface layer of cytoplasm is usually invisible. Hence, it is quite translucent and has refractive and dispersive powers very close to those of water. If the cell wall be cut across the surface cytoplasm shrinks. The chloroplasts either shrink or separate into rounded masses. The chloroplasts have a higher viscosity and elasticity than the gel in which they are imbedded.

The pyrenoid is a complex structure. Dissection shows the presence of an optically dense but fragile wall which, when broken, frees a globule that is of considerable interest. This globule shows many of the optical properties of an oil droplet but has too high a viscosity to round up under the influence of surface tension; therefore it seems to be a true gel.

None of the cytoplasm goes into solution very readily even when cut into very minute pieces.

The nucleus of *Spirogyra* is a gel that has higher viscosity and refractive and absorptive powers than the cytoplasm. It is also more cloudy than the cytoplasm. There are denser areas in the nuclear substance in the form of granules and threads that form a sort of network. Small pieces dissected from all parts of the nucleus

into water, not only do not go into the sol state but remain too rigid to show surface tension effects. Pieces of broken glass needles stick firmly to the nuclear gel when imbedded in it. The image of the nucleus is false in important details. The denser areas, when in the focal plane, appear as grayish or slightly opalescent granules and threads and when above or below the focal plane as dark spots and lines. Besides, if the intensity of the illumination be increased the network appears much finer. Very small dense masses of gel could be partly freed from the remaining nuclear substance. It seems proper to term such structures granules. On the other hand, the dense masses that produce the appearance of a network in the image are not actual threads that are sharply separated from the surrounding gel but irregularly shaped dense areas that grade into the immediately contiguous diluter gel. The light spots that change their position at different focal planes seem to be due chiefly to two factors, viz., a relatively low absorptive power of the gel occupying the interstices of the network and diffraction by the edges of the denser areas.

It seems certain that the vacuolar fluid of *Spirogyra* contains protein and must be considered a hydrosol. Much evidence has been adduced in support of this statement. A number of injections of Millon's fluid into the vacuole were made with positive results. Extremely small solid particles appeared in the cell sap after the injection of such precipitating agents for proteins, as saturated sublimate, 40 per cent formaldehyde, saturated picric acid and saturated phosphotungstic acid containing 5 per cent sulphuric acid.

The vacuolar fluid is cloudy. This is positive proof of the presence of ultramicroscopic particles which would ordinarily be considered protein even in the absence of a positive color test for protein.

The cell sap of *Chara* seems to be richer in protein than that of *Spirogyra*. This conclusion is based on the fact that a comparatively heavy precipitate results from the intravacuolar injection of saturated sublimate or 40 per cent formaldehyde. Hence, it is probable that cell sap containing protein is very common in plants.

*Mucor*, *Saprolegnia*, *Hydrodictyon*, *Chara* and the parenchymatous cells of the leaves of *Tradescantia* have been dissected for comparison with animal cells. In general, it may be stated that the cellulose walls of plants are extremely cohesive and are cut and punctured

with considerable difficulty. The protoplasm of plant cells is much more dilute or less rigid than that of animal cells.

RESTING AND DIVIDING MALE GERM CELLS OF THE SQUASH  
BUG (ANASA). GRASSHOPPERS AND CRICKETS

A brief note has been published on this subject.<sup>21</sup>

The whole cell substance of resting and dividing spermatogonia and spermatocytes is a moderately viscous gel. Cutting away pieces of the cytoplasm and nucleus in Ringer's fluid shows that these structures are far too rigid to flow or change shape under such experimental treatment. The appearance of a network is due to denser masses of nuclear gel that grade into the diluter surrounding substance. No definite threads or fibrils could be dissected out of resting nuclei. Some of the optical principles involved in a study of the living nuclei of spermatogonia and spermatocytes were discussed in connection with the nucleus of proteus.

Very definite statements can be made about the physical properties of chromosomes and spindle fibres. The chromosome has been found to be the most highly concentrated and rigid part of the nuclear gel. Such a mass of gel is less translucent and has a higher refractive index and absorptive power than the diluter homogeneous gel in which it is imbedded. A chromosome when dissected out shows no affinity for water and does not disintegrate readily. Pieces of it stick to the glass dissecting needle but when drawn out show no marked elasticity. The spindle fibre is an elastic concentrated thread of nuclear gel and its absorptive power and refractive index are also different from those of the diluter gel in which the spindle fibre is imbedded and from which it cannot be entirely freed. Metaphase spindle fibres that were dissected out with great care seemed continuous with the ends of the chromosomes. The homogeneous gel in which a telophase spindle is imbedded is so rigid, that all the surrounding cytoplasm can be cut away and the spindle and chromosomes show no appreciable change; metaphase, anaphase and telophase spindles can be cut to pieces in Ringer's fluid and the pieces are so rigid that they undergo no change in shape.

<sup>21</sup> KITE and CHAMBERS: 1912, *Science*, N. S., xxxvi, p. 639.



Many of the physical and chemical changes of cell-division are reversible. Pressure on the cell plate of spermatocytes in telophase has caused rapid fusion of the daughter cells and extensive swelling and loss in rigidity of the protoplasmic gel in which the spindle fibres are imbedded. If the displaced spindle fibres and chromosomes are dissected out, after such a partial reversal, they are found to have undergone no appreciable change in rigidity.

From a preliminary study of mitosis, a few conclusions, that are probably general, can be drawn. It seems that cell-division results primarily from concomitant shrinking and swelling or change in water-holding-power of different portions of the cell protoplasm. Many of the structural elements of the mitotic figure separate out of the protoplasm and change in rigidity according to their water-content. During the prophase, the nuclear substance becomes so soft that movement of the components of the nucleus is affected by flowing of the nuclear gel. The mechanism at the basis of this flowing seems to be a change in water-holding-power of the nuclear components.

I wish here to thank Dr. A. P. Mathews for the very helpful interest that he has shown in this investigation.

## ON THE NATURE OF THE IODINE-CONTAINING COMPLEX IN THYREOGLOBULIN.

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(Received for publication, January 27, 1913.)

In this paper are given the results of an attempt to determine the nature of the active complex in the iodine-containing active principle of the thyroid gland. Although the nature of this group was not determined, the quantitative physiological results here reported serve to establish certain predicted and other unexpected facts and to eliminate certain hitherto considered probabilities.

The problem was taken up both by analytical and by synthetic methods. In the former method the physiological activity and iodine content of the dried thyroid tissue, the globulin therefrom and various products of hydrolysis from this globulin were determined quantitatively. In the second method two iodized amino-acid derivatives, not previously tested by quantitative methods, were prepared synthetically and their physiological activity studied quantitatively.

In thus tracing the active complex a number of important assumptions were made. First, that the activity of unaltered thyroid tissue depends quantitatively on its iodine content. Second, that the best method known for measuring this activity directly and quantitatively is the Reid Hunt acetonitrile test.<sup>1</sup> Third, that in case the iodine is present in the products of hydrolysis in the same combination as in the globulin then, per unit of iodine, these will still possess an activity comparable with the original globulin. Fourth, that in case the iodine complex is an iodized amino-acid and that in case this is decomposed in the process of hydrolysis then the synthetic preparation of various iodized amino-acids or derivatives thereof and the quantitative testing of

<sup>1</sup> This *Journal*, i, p. 33, 1905.

these per unit of iodine may determine the probable nature of the iodine complex. In other words, the actual quantitative physiological activity per unit of iodine as measured by the Reid Hunt method was taken as the crucial test for the presence or absence of the unaltered iodine complex.

The historical development of the relation of thyroid activity to iodine content need not be considered at this time, especially in view of the thorough reviews and extensive confirmatory experiments made by Reid Hunt and A. Seidell,<sup>2</sup> as well as the comparative histological and chemical studies by Marine in coöperation with Lenhardt and Williams.<sup>3</sup> A careful study of these papers justifies the first assumption. The second assumption is also well taken provided the proper precautions are observed as shown by Reid Hunt and A. Seidell.<sup>4</sup> Other methods for testing the physiological activity of thyroid substance, based on changes in blood pressure,<sup>5</sup> on increasing the irritability of the depressor nerve,<sup>6</sup> on changes in nitrogen metabolism<sup>7</sup> and on curative effects in cretinism<sup>8</sup> have been employed, but are not applicable in a quantitative study, nor are they as specific reactions.

Of the third and fourth assumptions we had no definite proof. The studies of Oswald<sup>9</sup> and others show that during hydrolysis of thyreoglobulin only 30 per cent or less of the iodine remains in organic combination. The iodine thus combined is in the various fractions and qualitatively it has been determined<sup>10</sup> that probably the greater activity remains in the more complex products

<sup>2</sup> Bulletins 47 (1908) and 69 (1910) of the Hygienic Laboratory, U. S. Public Health and Marine Hospital Service.

<sup>3</sup> *Johns Hopkins Hospital Bull.*, xviii, p. 359, 1907; *Journ. Inf. Dis.*, iv, p. 417, 1907; *Archives of Internal Med.*, i, p. 349, 1908; *Ibid.*, iii, p. 66, 1909; *ibid.*, iv, p. 440, 1909; *ibid.*, vii, p. 506, 1911; *ibid.*, viii, p. 265, 1911; *Journ. of Exp. Med.*, xiii, p. 455, 1911.

<sup>4</sup> *Loc. cit.*; *Journ. of Pharmacol. and Exp. Ther.*, ii, p. 15, 1910.

<sup>5</sup> von Fürth and Schwarz: *Pflüger's Archiv*, cxxiv, p. 113, 1908.

<sup>6</sup> von Cyon and Oswald: *Pflüger's Archiv*, lxxxiii, p. 199, 1901; Asher and Flack: *Zeitschr. f. Biol.*, lv, p. 83, 1910.

<sup>7</sup> Baumann: *Zeitschr. f. physiol. Chem.*, xxi, p. 487, 1896; *ibid.*, xxii, p. 1, 1896; *Münch. med. Wochenschr.*, xl, 1896.

<sup>8</sup> E. Pick and F. Pineles: *Zeitschr. f. exp. Path. u. Ther.*, vii, p. 518, 1909-10.

<sup>9</sup> *Arch. f. exp. Path. u. Pharm.*, lx, p. 115, 1908.

<sup>10</sup> Pick and Pineles: *loc. cit.*



of hydrolysis where also the greater part of the organically combined iodine is found. What relation the activity bears to the iodine content therein has however not been determined. As stated above we have evidence that some of the iodine is split off as iodide, but we have no direct evidence that all the organically combined iodine found in the products of hydrolysis is still in the same complex or in the same structural relationship as in the original thyreoglobulin. A number of iodized amino-acids have been studied qualitatively as to physiological activity. In no case has thyroid activity been detected. The most conclusive results as to the inactivity of 3,5-iodo-laevo-tyrosine are those reported by Strouse and Voegtlin.<sup>11</sup> Other observations on the inactivity of various iodized proteins, which on hydrolysis yield 3,5-iodo-tyrosine, also bear out these conclusions. The studies on other iodized amino-acids do not lead to definite conclusions. Thus von Fürth and Schwarz<sup>12</sup> prepared and studied what they considered iodized phenylalanine, histidine and tryptophane. They reported all these substances as physiologically inactive, but gave no data indicating that they had really separated iodo-derivatives of these substances. Pauly<sup>13</sup> however actually separated pure tetra-iodohistidine anhydride and tri-iodo-imidazol and reported that these substances increased the respiratory and pulse frequencies, although uniodized imidazol had no such action. These considerations lead us to conclude that for the present the validity of the third and fourth assumptions is unknown to us and that the true answers thereto are part of the problem in hand.

#### EXPERIMENTAL PART.

The mode of attack has already been outlined above. The details as to the methods employed and the preparation of the substances studied are given below.

##### *A. Preparations.*

*Dried hog thyroids.* Hog thyroids<sup>14</sup> were freed mechanically from fat as much as possible and dried on glass plates in a current of air at 30-35°C.

<sup>11</sup> *Journ. of Pharm. and Exp. Ther.*, i, p. 123, 1909.

<sup>12</sup> *Pflüger's Archiv*, cxxxiv, p. 113, 1908.

<sup>13</sup> *Ber. d. deutsch. chem. Gesellsch.*, xliii, p. 2243, 1910.

<sup>14</sup> The raw material for this research was supplied by the Armour Laboratory Department.

The mass was then ground to a coarse powder and fat removed by ether in the cold. The remaining dry mass was then finely powdered. Duplicate determinations on this gave 0.243 and 0.250 per cent iodine.

*Thyreoglobulin.* This was prepared as previously described.<sup>15</sup> Duplicate determinations on this gave 0.462 and 0.468 per cent iodine.

*Iodothyryn (a)* was prepared by the usual Baumann process from the above thyreoglobulin. The extraction with 95 per cent alcohol of the melanoidin precipitate was made in a continuous hot extractor. Duplicate determinations gave 5.81 and 5.85 per cent iodine.

*Iodothyryn (b)* was obtained in the same way from the melanoidin precipitate which separated in the complete hydrolysis of some of the same thyreoglobulin by 30-35 per cent sulphuric acid. This on analysis gave 7.51 per cent iodine.

*Iodothyryn (c)* was obtained from 40 grams of the same globulin by hydrolysis for three days at room temperature and for twenty-four hours at boiling temperature with 20-25 per cent phosphoric acid. Phosphoric acid was used as it was thought that possibly the oxidative action of sulphuric acid might have an injurious effect. This amount of globulin yielded 3.30 grams of melanoidin, containing 1.75 per cent iodine. The iodothyryn extracted from this represented 24 per cent of the weight and contained 4.44-4.46 per cent iodine. Thus only 61 per cent of the iodine in the melanoidin fraction was recovered in the alcohol extract.

*Metaprotein (A<sub>4</sub>).* The filtrate from the melanoidin fraction above was neutralized with NaOH and the metaprotein separated and dried over sulphuric acid in a vacuum desiccator. This weighed 1.62 grams and contained 1.51-1.53 per cent iodine.

*Primary albumose (A<sub>5</sub>).* The filtrate from above was half saturated with zinc sulphate after slightly acidifying with sulphuric acid. The precipitate obtained was dialyzed until free from sulphate. In this fraction there were recovered 3.1 grams containing 0.22-0.225 per cent iodine.

*Secondary albumose (A<sub>6</sub>).* Obtained from the filtrate from above by complete saturation with zinc sulphate. The precipitate after dialyzing as above yielded 4 grams dry substance containing 0.069 per cent iodine.

The table (I) below gives a summary of the distribution of iodine in the different fractions above.

TABLE I.

	WEIGHT RECOVERED	PER CENT OF IODINE THEREIN	WEIGHT OF IODINE	PER CENT OF TOTAL IODINE IN THE GLOBULIN
Melanoidin precipitate	3.30	1.74	0.0575	30.9
Metaprotein.....	1.62	1.52	0.0246	13.2
Primary albumose....	3.01	0.22	0.0066	3.5
Secondary albumose...	4.0	0.0695	0.0027	1.5
Undetermined iodine..				50.9

<sup>15</sup> This *Journal*, ix, p. 121, 1911.



*Phosphotungstic acid precipitate.* Another 40 grams of thyreoglobulin were boiled with 25 per cent phosphoric acid for ninety-three hours. The filtrate from the melanoidin precipitate and metaprotein, after removal of the phosphoric acid by  $\text{Ba}(\text{OH})_2$  and the excess of barium by sulphuric acid, was concentrated under diminished pressure to about 250 cc. This was then freed from proteose and peptone by the Kutscher tannin method.<sup>16</sup> The filtrate finally obtained here after removal of the excess of lead was boiled with  $\text{BaCO}_3$  to remove the ammonia. The dissolved barium was again removed by sulphuric acid. The filtrate after acidifying with  $\text{H}_2\text{SO}_4$  to 5 per cent strength was precipitated with phosphotungstic acid in the usual way. The precipitate after thorough washing with 2.5 per cent phosphotungstic acid solution was freed from phosphotungstic acid, barium and sulphate in the usual way. Duplicate determinations on the dry amino-acid mixture gave 0.0107 per cent and 0.0093 per cent iodine.

Another phosphotungstic acid precipitate from a hydrolysis by  $\text{H}_2\text{SO}_4$  was worked up in the same way. This dry residue contained 0.0068 per cent iodine. The two samples were mixed and designated as P.T.A. Ppt. 1. This mixture contained 0.0073 per cent iodine.

*Phosphotungstic acid filtrate (1).* This was freed from phosphotungstic acid in the usual way. The amino-acid solution was evaporated to dryness. Duplicate determinations on the dry amino-acid mixture gave 0.0024 per cent iodine.

*Phosphotungstic acid precipitate (2).* This was obtained in the same way as the above from the partial hydrolysis by 10 per cent sulphuric acid of 141.6 grams of thyreoglobulin containing 0.511 per cent iodine. The purified dry residue by analysis contained 0.0043 per cent iodine.

*Phosphotungstic acid filtrate (2).* The filtrate from the above was treated in the usual way. The dry purified amino-acid mixture left gave in duplicate determinations 0.0045 and 0.0043 per cent iodine.

*Tetra-iodohistidine anhydride.* Histidine was prepared from ox erythrocytes by the method of Frankel.<sup>17</sup> Various methods were employed in trying to iodize the dichloride or the base itself but in no case were there indications of true absorption of iodine, but rather decomposition of the histidine. While this work was under way Pauly<sup>18</sup> published his observations with the same conclusions as to the difficulty or inability to iodize histidine directly. At the same time, as stated above, he published his observations on tetra-iodohistidine anhydride. Following the methods given by Pauly<sup>19</sup> the preparation of the methyl ester of histidine dichloride was carried out and from this the histidine anhydride by the Pauly modification<sup>20</sup> of the Fischer and Zuzuki method. The histidine anhydride was recrystallized from hot water a number of times to obtain the more

<sup>16</sup> *Zentralbl. f. Physiol.*, xix, p. 504, 1905.

<sup>17</sup> *Monatsh. f. Chem.*, xxiv, p. 230, 1903.

<sup>18</sup> *Ber. d. deutsch. chem. Gesellsch.*, xliii, p. 2243, 1910.

<sup>19</sup> *Zeitschr. f. physiol. Chem.*, lxiv, p. 75, 1910.

<sup>20</sup> *Loc. cit.*



readily soluble laevorotatory form. This was then iodized according to the Pauly method. One determination on the snow-white product gave 63 per cent iodine (theoretical 65 per cent). The slightly lower value may be due to an admixture of a small amount of di-iodohistidine anhydride.

*Iodized tryptophane.* Tryptophane was prepared from commercial casein by the Hopkins-Cole method.<sup>21</sup> Several attempts were made to iodize the pure crystals by the method of Neuberg,<sup>22</sup> but in no case was a substance obtained containing more than 6.3 per cent iodine. The preparation finally made for physiological testing was obtained by dissolving one milligram molecule of tryptophane in 4 cc. of  $\frac{N}{2}$  NaOH, cooling by immersing in ice water and, while keeping cool and stirring well, adding drop by drop 6 cc. of aqueous  $N$  iodine solution. The mixture was allowed to stand at ice box temperature for twenty-four hours, then filtered off. The precipitate was well washed with cold water and dried over sulphuric acid in a vacuum desiccator. The product obtained is light brown in color, readily soluble in alkalis, reprecipitated on acidifying and liberates a very small amount of iodine to chloroform on shaking therewith. Duplicate determinations on this gave 41.5 and 41.9 per cent iodine (the theoretical for mono- and di-iodo-tryptophane are 38.4 per cent and 55.7 per cent respectively).

#### B. Methods.

*Determination of iodine.* The Hunter<sup>23</sup> method with slight modifications was employed. The material to be analyzed, taken in quantities of 0.05–2 grams was mixed with 15 grams of fusion mixture and covered with 10 grams of fusion mixture as suggested by Hunter. To conduct the fusion the Roger's ring burner was found to be much more satisfactory in ensuring a uniform rapid heating without overheating. With the size of the flame once determined one finds ten minutes to be ample time to give a satisfactory, easily removable fusion. In the treatment with alkaline hypochlorite it was considered best to warm to 40°C. for ten minutes. In acidifying it is very important to make sufficiently acid and then always to the same degree. Sulphuric acid of 25 per cent strength was used here and since the same amounts of fusion mixtures and hypochlorite were used in each case the acidity was well controlled by always adding the same amount of acid. In removing the excess of chlorine gentle boiling was continued for forty minutes after the negative test of the vapors by starch iodine paper. In this way the blank test on the reagents never was more than 0.1 cc. of a  $\frac{N}{200}$   $Na_2S_2O_3 \cdot 5H_2O$  solution.

*Physiological testing by the Hunt method.* The method employed was that of feeding the same quantity of iodine, in the different combinations, to white mice in such a manner as to make as certain as possible the entire consumption of the material fed. In order to do this each mouse was first

<sup>21</sup> *Journ. of Physiol.*, xxvii, p. 418, 1901.

<sup>22</sup> *Biochem. Zeitschr.*, vi, p. 276, 1907.

<sup>23</sup> *This Journal*, vii, p. 321, 1910.

fed for three or four days with cracker dust made into pellets of known weight. At the close of this preliminary feeding the unconsumed material was weighed and from this the average amount eaten per day determined. For ten days following this period each mouse then received this weight of cracker dust, with the incorporated iodine-containing substance, in the form of pellets. The control mice were fed in the same way with plain cracker dust pellets. At the end of the 10-day feeding period the acetonitrile was injected subcutaneously. Each dose administered in series I, II and III was contained in 1 cc. of fluid; in series IV-IX, in 0.5 cc.; in series X, in 0.66 cc. In most cases the animals consumed the food very well. All the mice used were raised in the laboratory building on a diet of milk and crackers with occasional bits of lettuce until used for the experiment. Care was taken to compare mice of as nearly the same age as possible. In the tables below the litter number of each mouse is given. The ages of the mice of the various litters were as follows: Litter 2, 119 days; litter 3, 102 days; litter 4, 100 days; litter 5, 80 days; litters 6 and 10, 99 and 113 days respectively; litter 9, 115 days; litters 11-12, 125 and 135 days respectively; litters 13-14, 144 and 151 days respectively; litter 28, 85 days; litters 29-30, 59 and 66 days respectively; litters 31, 32 and 34, 95, 85 and 97 days respectively; litters 33-35, 101 and 89 days respectively; litters 36-37, 89 days; litter 38, 79 days; litters 56-60, 91-103 days.

### C. Discussion of the physiological tests.

*Thyreoglobulin.* Series I shows that thyreoglobulin possesses the full activity per unit of iodine when compared with the dried thyroid from which it was prepared. This is also confirmed by series IV where a decomposition product obtained from the globulin still shows the complete activity per unit of iodine. The whole of the physiological activity of the gland is therefore quantitatively in the thyreoglobulin.

*Metaprotein.* As stated above, this still shows the full activity per unit of iodine although the percentage concentration of iodine has increased from 0.465 per cent in the thyreoglobulin to 1.52 per cent in the metaprotein.

*Iodothyrim.* None of the iodothyrim preparations tested was found to bring about a resistance to acetonitrile more than three-fourths of that produced by the thyroid-tissue fed mice. The indications are that these preparations are all about equally inactive. Iodothyrim is therefore less active per unit of iodine than the thyreoglobulin. See series III and V.

*Primary albumose.* This is still very active, as shown by series IV and VII; although the full activity per unit of iodine is not



shown to be present in every case tested. In this connection it may be mentioned that the results in series VI are of no value. This series VI, however is an illustration of irregular results, due in all probability to impure acetonitrile. The acetonitrile was taken from a freshly opened bottle and found to smell decidedly of hydrocyanic acid. Before using, it was shaken twice with saturated potassium carbonate solution, dehydrated with  $P_2O_5$  and twice distilled from fresh  $P_2O_5$ . Finally it was redistilled and the fraction collected between 79 and 83°C. This distillate was used in series VI. For the later series this distillate was again purified in the same way three times and finally redistilled twice without the addition of  $P_2O_5$ . Here the distillate was collected between 80.5 and 81.5°C.

*Secondary albumose.* This is much less active per unit of iodine than either the iodothyron preparations or the primary proteoses. Series VII shows this, where the maximum dose resisted is only 40 per cent of the maximum dose resisted by the thyroid-tissue fed mice.

*Amino-acids from the phosphotungstic acid precipitate and the phosphotungstic acid filtrate respectively.* The results in series IX indicate that the former possess very little physiological activity as measured by the Hunt method. On the whole, however, the results here are very unsatisfactory as the mice did not eat the amino-acid mixtures well, there being two or more days' feeding left. The results indicate that these amino-acid fractions contain very little thyroid activity. This is better shown in series X where only one-tenth the quantity of iodine-containing substances was fed. Although the mice fed with dried thyroid tissue resisted an amount over two and a half times that of the control mice, still the mice fed with the same amount of iodine, but in the form of amino-acids, resisted very little, if any, more of the acetonitrile than the control mice. In other words, these amino-acid fractions show a very slight physiological activity, if indeed they possess any activity whatever.

*Tetra-iodohistidine anhydride and iodotryptophane.* These substances when fed in amounts representing ten times the amount of iodine fed as thyroid tissue do not appreciably increase the resistance to acetonitrile. See series II and VIII.



Table II gives a summary of the results above. The relative physiological activity is expressed (on the basis of feeding the same amount of iodine in each case) as follows: representing in each case, by 100, the largest dose of acetonitrile from which the thyroid-tissue fed mice recovered, then the other figures represent the proportions the limiting doses of the otherwise fed mice bear thereto.

TABLE II.

	RELATIVE ACTIVITY	IODINE IN THE SUBSTANCE	TOTAL IODINE IN THE TISSUE
		<i>per cent</i>	<i>per cent</i>
Thyroid tissue.....	100	0.247	100.0
Thyreoglobulin.....	100	0.465	100.0
Metaprotein.....	100	1.520	13.2
Iodothyrim.....	50-75	4.46-7.51	18.3
Primary albumose.....	80-100	0.220	3.5
Secondary albumose.....	40	0.0695	1.5
Amino-acids precipitated by phosphotungstic acid.....	0(+?)	0.0043	
Amino-acids not precipitated by phosphotungstic acid.....	0(+?)	0.0044	
Tetra-iodohistidine anhydride.....	0	65.00	
Iodotryptophane.....	0	41.70	

These results show that both the thyroid activity and iodine may be concentrated from thyroid tissue in the thyreoglobulin as well as in the metaprotein and iodothyrim from the latter. Per unit of iodine, however, we have full activity retained in the thyreoglobulin and metaprotein only. In the primary albumose fraction we have a lowering in the percentage concentration of iodine and also a slight lowering in the physiological activity per unit of iodine. In the secondary albumose this is still more marked. In the amino-acid fractions the activity is extremely low if present. In view of the researches of Hunt and Seidell with various iodine compounds and in view of the results obtained here, we cannot attribute the protective action in any of these cases to iodine itself, but to a specific iodine-containing complex in the thyreoglobulin. It is significant to note that the highest physiological activity per unit of iodine is found in the original protein and in the more complex products of hydrolysis. Since the lowest products of hydrolysis are still less active per unit of iodine than the secondary albu-

mose it indicates either that the iodine group is altered in the hydrolysis, or that the iodine-containing group when in simpler combination or when separated, does not possess the full specific thyroid activity. That the iodine-containing group when once separated would not possess the full activity is not at all unlikely, but we would be inclined to expect it to show some activity; at least when given in amounts such as were employed by Strouse and Voegtlin with iodotyrosine and by the author in the experiments with tetra-iodohistidine anhydride and iodotryptophane. The indications as to the presence of tyrosine and tryptophane in iodothyryn are very favorable, both from the chemical studies on iodothyryn and also from similar studies on iodine-free melanoidins.<sup>24</sup> It is not likely that the iodine is split off and then later added to the melanoidin fraction; it is more likely that it is already present in the globulin in the melanoidin-forming groups and remains in the original position in these groups, but that the groups themselves are changed in regard to each other and thus the activity affected to some extent; a poly-iodo derivative may be changed to a mono-iodo derivative and then may show decided differences in physiological activities. If this were not the case we would expect artificially iodized melanoidins to show a decided thyroid activity. Furthermore, it is not likely that sufficient hydriodic acid is split off in the early stages of the hydrolysis to yield as much iodine as is contained in the melanoidin fraction. Finally, it is not at all improbable that we here have to do with a specific iodophore group just as in hemoglobin we have the chromophore group containing the iron. The negative results with artificially iodized proteins speak strongly in favor of this view.

#### CONCLUSIONS.

1. The full activity of thyroid tissue is contained in the thyreoglobulin fraction when this activity is measured by the Hunt method.
2. The full activity per iodine unit is still present in the metaprotein fraction from this globulin, although the iodine content in the metaprotein fraction has been increased over threefold that of the globulin itself.

<sup>24</sup> Samuely: *Hofmeister's Beiträge*, ii, p. 355, 1902.



3. The other products of the hydrolysis studied, primary albumose, iodothyron and secondary albumose, show a gradual decrease in activity per unit of iodine in the order given.

4. The amino-acid fractions precipitated and not precipitated by phosphotungstic acid from the partially hydrolyzed thyroglobulin still contain very small amounts of iodine and per unit of iodine are either extremely low in activity or entirely inactive.

5. Tetra-iodohistidine anhydride and iodotryptophane do not possess thyroid activity as determined by the Hunt method.

I wish to express my thanks to Prof. A. P. Mathews for suggestions made in the course of the work.



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## SERIES I. February 25-March 8.

MOUSE	LITTER NO.	FED DAILY WITH CRACKER DUST PLUS	FATAL DOSE OF ACETO- NITRILE	DEATH OCCURRED AFTER	DOSE OF ACETO- NITRILE FROM WHICH RECOVERY OCCURRED
			mg. per gm.	hrs.	mg. per gm.
(a) ♂.....	4		0.4	1 $\frac{1}{4}$	
(b) ♀.....	4				0.35
(c) ♀.....	4				
(d) ♀.....	4		Gravid, not injected		
			Escaped from cage		
(e) ♀.....	4	1 mg. dried hog thy- roid (=0.00247 mg. I)	4.49	3 $\frac{1}{2}$	
(f) ♂.....	3		died while feeding		
(g) ♂.....	3		4.0	30	
(h) ♀.....	3	0.531 mg. thyreo- globulin (=0.00247 mg. I)	4.0	1 $\frac{1}{2}$	
(i) ♀.....	3				3.79
(j) ♀.....	3				3.50

## SERIES II. April 21-May 1.

(a) ♂.....	3		0.55	4 $\frac{1}{4}$	
(b) ♂.....	5				0.30
(c) j (Ser.I)...	3				0.40
(d) i (Ser.I)...	3				0.45
(e) ♂.....	2	1 mg. dried hog thy- roid (=0.00247 mg. I)	5.0	3 $\frac{1}{2}$	
(f) ♂.....	2		4.0	4	
(g) ♂.....	2		4.5	23	
(h) ♀.....	4	0.00392 mg. tetra- iodohistidine an- hydride (=0.00247 mg. I)			0.55*
(i) ♀.....	4		0.60	<1 $\frac{1}{4}$	
(j) ♀.....	4		0.55	8 $\frac{1}{2}$	
(k) ♂.....	3	0.0392 mg. tetra- iodohistidine an- hydride (=0.0247 mg. I)	0.55	5	
(l) ♀.....	4		3.55	48	
(m) b (Ser.I)...	4				0.60

## SERIES III. May 19-29.

(a) ♂.....	6-10		0.30	5†	
(b) ♀.....	6-10				0.45
(c) ♂.....	9		died while feeding		
(d) ♂.....	9		0.50	8	

\* Slight loss in injection.  
† Not well when injected.

## SERIES III—Continued.

MOUSE	LITTER NO.	FED DAILY WITH CRACKER DUST PLUS	FATAL DOSE OF ACETO- NITRILE	DEATH OCCURRED AFTER	DOSE OF ACETO- NITRILE FROM WHICH RECOVERY OCCURRED
			mg. per gm.	hrs.	mg. per gm.
(e) ♂.....	9	1 mg. dried hog thy- roid (=0.00247 mg. I)	3.5	7	2.9
(f) ♀.....	6-10				
(g) ♂.....	6-10		3.2	30	
(h) ♂.....	9	0.0424 mg. iodothy- rin (a) (=0.00247 mg. I)	2.0	8	1.5
(i) ♀.....	6-10		3.0	2	
(j) ♂.....	6-10				
(k) ♂.....	9	0.0329 mg. iodothy- rin (b) (=0.00247 mg. I)	<2.5	2	
(l) ♀.....	6-10		died while feeding	died while feeding	
(m) ♂.....	6-10		died while feeding	died while feeding	
(n) ♀.....	6-10	0.0554 mg. iodothy- rin (c) (=0.00247 mg. I)	died while feeding	died while feeding	1.5
(o) ♀.....	6-10		2.5	8	
(p) ♀.....	6-10				

## SERIES IV. June 19-29.

(a) ♂.....	11-12	1 mg. dried hog thy- roids (=0.00247 mg. I)	3.0	3	2.5
(b) ♀.....	11-12				
(c) ♂.....	11-12		died while feeding	died while feeding	
(d) ♀.....	11-12				
(e) ♂.....	11-12	0.163 mg. metapro- tein (A <sub>4</sub> ) (=0.00247 mg. I)			2.0
(f) ♀.....	11-12				2.5
(g) ♀.....	11-12		3.0	3	
(h) ♀.....	11-12	{ 1.123 mg. primary albumose (A <sub>5</sub> ) (=0.00247 mg. I)	died while feeding	died while feeding	2.5
(i) ♀.....	11-12				

## SERIES V. August 2-12.

(a) ♂.....	13-14	1 mg. dried hog thy- roid (=0.00247 mg. I)			2.5
(b) ♂.....	13-14		died while feeding	died while feeding	
(c) ♀.....	13-14				<3.0*
(d) ♀.....	13-14	0.0424 mg. iodothy- rin (a) (=0.00247 mg. I)	2.8	3	2.0
(e) ♂.....	13-14		died while feeding	died while feeding	
(f) ♀.....	13-14				

\* Slight loss in injection.

## SERIES V—Continued.

MOUSE	LITTER NO.	FED DAILY WITH CRACKER DUST PLUS	FATAL DOSE OF ACETO- NITRILE  <i>mg. per gm.</i>	DEATH OCCURRED AFTER  <i>hrs.</i>	DOSE OF ACETO- NITRILE FROM WHICH RECOVERY OCCURRED  <i>mg. per gm.</i>
(g) ♀ .....	13-14	{ 0.0556 mg. iodothy- rin (c) (=0.00247 mg. I)	died while feeding		
(h) ♀ .....	13-14		2.5	3½	

## SERIES VI. November 24–December 4.

(a) ♀ .....	29-30	{ 1 mg. dried hog thy- roids (=0.00247 mg. I)	2.5	< 24	
(b) ♂ .....	29-30		3.0	1½	
(c) ♂ .....	29-30		2.0	< 18	
(d) ♂ .....	29-30	{ 1.123 mg. primary albumose (A <sub>5</sub> ) (=0.00247 mg. I)	died while feeding		
(e) ♀ .....	28		2.0	36-40	
(f) ♂ .....	29-30				2.5
(g) ♂ .....	28	{ 3.55 mg. secondary albumose (A <sub>6</sub> ) (=0.00247 mg. I)	1.5	< 4	
(h) ♀ .....	28		2.0	24-36	
(i) ♂ .....	28		1.25	20-36	

## SERIES VII. January 13-23.

(a) ♀ .....	31-34	{ 1 mg. dried hog thy- roid (=0.00247 mg. I)	died while feeding		
(b) ♂ .....	31-34				2.0
(c) ♀ .....	31-34				2.5
(d) ♂ .....	31-34				3.0
(e) ♂ .....	31-34	{ 1.123 mg. primary albumose (A <sub>5</sub> ) (=0.00247 mg. I)			2.5
(f) ♀ .....	31-34				2.0
(g) ♀ .....	31-34		2.8	> 6	
(h) ♀ .....	31-34		3.0	> 8	
(i) ♂ .....	31-34	{ 3.55 mg. secondary albumose (A <sub>6</sub> ) (=0.00247 mg. I)	2.0	2½	
(j) ♂ .....	31-34		1.2	> 4	
(k) ♀ .....	31-34		1.0	24	
(l) ♀ .....	31-34				1.2



SERIES VIII. *February 17-27.*

MOUSE	LITTER NO.	FED DAILY WITH CRACKER DUST PLUS	FATAL DOSE OF ACETO- NITRILE	DEATH OCCURRED AFTER	DOSE OF ACETO- NITRILE FROM WHICH RECOVERY OCCURRED
			<i>mg. per gm.</i>	<i>hrs.</i>	<i>mg. per gm.</i>
(a) ♀ .....	33-35				0.45
(b) ♀ .....	33-35				0.40
(c) ♀ .....	33-35				0.35
(d) ♀ .....	33-35		0.55	<18	
(e) ♂ .....	33-35	1 mg. dried hog thy- roids (=0.00247 mg. I)	4.0	2	2.0
(f) ♂ .....	36-37		died while	feeding	
(g) ♀ .....	36-37		3.0	6	
(h) ♀ .....	36-37				
(i) ♂ .....	33-35	0.0059 mg. iodotryp- tophane (=0.00247 mg. I)	0.55	>36	
(j) ♂ .....	33-35		1.6	2½	
(k) ♀ .....	36-37		1.0	18	
(l) ♀ .....	36-37		0.45	>24	
(m) ♂ .....	33-35	0.059 mg. iodotryp- tophane (=0.0247 mg. I)	1.0	< 3	0.5
(n) ♂ .....	33-35				
(o) ♀ .....	33-35		0.70	<18	

SERIES IX. *March 12-22.*

(a) a Ser. VIII	33-35	1 mg. dried hog thy- roid (=0.00247 mg. I)	4.0	< 6	3.5 3.0
(b) b Ser. VIII	33-35				
(c) c Ser. VIII	33-35		did not	eat; not	
(d) n Ser. VIII	33-35				
(e) ♀ .....	38	33.6 mg. P. T. A. Ppt. 1 (=0.00247 mg. I)	died while	feeding	0.8*
(f) ♀ .....	38		died while	feeding	
(g) ♂ .....	38				
(h) ♂ .....	38	100 mg. P.T.A. Filt.	1.0	< 3*	
(i) ♂ .....	38	1 (=0.0024 mg. I)	0.8	<18*	
(j) ♂ .....	38		0.6	<18*	

\* Two or more days feeding left. This experiment is not reliable as animals were used which had recovered in previous experiments and the differences in age were too great for such young animals.

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## SERIES X.

MOUSE	LITTER NO.	FED DAILY WITH CRACKER DUST PLUS	FATAL DOSE OF ACETO-NITRILE	DEATH OCCURRED AFTER	DOSE OF ACETO-NITRILE FROM WHICH RECOVERY OCCURRED
			mg. per gm.	hrs.	mg. per gm.
(a) ♂.....	56-60		0.5 died while feeding	<10	
(b) ♂.....	56-60				
(c) ♀.....	56-60				0.4
(d) ♂.....	56-60				0.35
(e) ♂.....	56-60	0.1 mg. dried hog thyroid (=0.000247 mg. I)	1.2	3½	
(f) ♂.....	56-60				1.1
(g) ♀.....	56-60				1.0
(h) ♀.....	56-60				not injected, gravid
(i) ♂.....	56-60	5.74 mg. P. T. A. Ppt. 2 (=0.000247 mg. I)	0.5 1.0 0.8	48* < 6† < 16	
(j) ♂.....	56-60				
(k) ♂.....	56-60				
(l) ♀.....	56-60				0.4
(m) ♂.....	56-60	5.61 mg. P. T. A. Filt. 2 (=0.000247 mg. I)	1.0 0.8 0.7	3½ <12 24	
(n) ♂.....	56-60				
(o) ♂.....	56-60				
(p) ♂.....	56-60				0.5

\* Two days' feeding left.

† About one day's feeding left.

## CONTRIBUTIONS TO THE CHEMICAL DIFFERENTIATION OF THE CENTRAL NERVOUS SYSTEM.

### I. A COMPARISON OF THE BRAIN OF THE ALBINO RAT AT BIRTH WITH THAT OF THE FETAL PIG.

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(Received for publication, February 20, 1913.)

#### INTRODUCTION.

For the study of the progressive changes in the central nervous system during growth and senescence, the albino rat, on account of its small size, short span of life and its powers of rapid reproduction<sup>1</sup> is especially suited. Its growth processes are, moreover, strikingly like those of man, as has been brought out by the extensive investigations of Dr. Donaldson within the past few years. It was, therefore, decided to use this animal for a study of the chemical differentiation of the central nervous system during growth.

The youngest brains which could be conveniently collected for chemical analysis were those of rats just born. As it was not certain that the rat at this period of development was sufficiently immature (chemically undifferentiated) to serve as the starting point for such a growth series, it was suggested by my brother, Dr. Waldemar Koch, that the brain of the new born rat be compared chemically with the brain of the fetal pig, collected at various stages of fetal life.

By such a comparison we hoped to determine the physiological age of the rat at birth in terms of fetal pig material; and to obtain, possibly, from the pig fetus, material which would be more immature than the new born rat.

<sup>1</sup> H. H. Donaldson: President's Address, *Journ. of Nervous and Mental Disease*, xxxviii, p. 258, 1911.



## MATERIAL AND METHODS.

The rat material was obtained from the Wistar Institute of Anatomy, which supplied the brains of rats of known age, from animals which had been raised under constant conditions; two factors which are absolutely essential for such a study. The material was collected by Dr. Hatai and the method used was that adopted by the Institute. This in brief is as follows: the rat was chloroformed; the skull opened from the dorsal side; the division between the brain and the cord made at the tip of calamus scriptorius; and the brain removed. The meninges of the brain were left intact. Such blood as it contained was, therefore, included in the weight. Immediately after removal the brain was placed in a closed weighing bottle, quickly weighed to within 10 mgms.<sup>2</sup> and transferred to a wide mouthed bottle of 300 cc. capacity containing absolute alcohol. The weighing bottle was weighed back and the difference recorded as the weight of the sample. As the weight of one brain from rats at this early age is 0.2 — 0.3 gram and as it takes at least from 25 to 50 grams to make one sample for analysis, a large number of brains had to be collected (100 brains of the rat at birth for one 25-gram sample). As this covered a period of several weeks it was necessary to heat the sample from time to time in a water bath kept at a temperature of 70°C. to insure a thorough penetration of the alcohol and sterilization. The amount of alcohol was so adjusted as to make the final concentration not less than 80–85 per cent. A well fitting cork stopper covered with tin-foil was now inserted and the bottle carefully shaken to insure a uniform mixture. The dates of collection of the samples were recorded, as the time a sample has been kept in some cases influences the analytical results.<sup>3</sup> The tightly corked bottles were then shipped to the Laboratory of Biochemistry of the University of Chicago, where the samples were analyzed according to Koch's methods of tissue analysis.<sup>4</sup>

<sup>2</sup> A coarse weighing of the brain was permissible in this instance as it was not the exact brain weight that was sought but merely data for indicating roughly when the required amount of material had been obtained.

<sup>3</sup> W. Koch: Methods for the Quantitative Chemical Analysis of Animal Tissue, *Journ. of the Amer. Chem. Soc.*, xxxi, p. 1340, 1909.

<sup>4</sup> *Ibid.*, pp. 1329–64.

The fetal pig material was collected by my brother at the Chicago Stock Yards. The fetuses selected were 50, 100, and 200 mm. in length. The pregnant uterus was opened: and the fetuses removed. The neck-rump length of each litter was taken and if the average length corresponded to one of the three sizes mentioned above, the entire litter was taken, placed upon ice and in this chilled condition taken to the laboratory, where the brains were immediately removed, preserved and later analyzed according to the same methods used for the rat material.

#### RESULTS OF ANALYSES.

The results from the chemical analysis of the brains from the new born rat and the adult rat are given in table I: those of the 50, 100, and 200 mm. pig fetuses are recorded in table II, and table III gives the summary of all the averages which have been taken from the figures which were most consistent. The brain of the 200 mm. pig fetus was plainly more differentiated, it is therefore left out of table III and of the final discussion of results.

#### DISCUSSION OF CHEMICAL RESULTS.

Before taking up a comparison of the new born rat with the pig fetus it may be well to state, briefly, the chief chemical changes in nervous tissue during growth. It is well known that the chemical composition of a tissue varies with age and that the water content is one of the most important variables. Donaldson states that, "the progressive diminution of the percentage of water in the brain is a function of age and is not significantly modified by any conditions to which the animals have been thus far experimentally subjected."<sup>5</sup> He suggested that this "is to be regarded as an index of fundamental chemical processes, which take place in the more stable constituents of the nerve cells."<sup>6</sup> The principal chemical differences due to growth, noted by my brother, are, "a decrease in moisture, proteins, extractives, and ash as the brain increases with age, and an increase in cerebrosides, sulphatides,

<sup>5</sup> H. H. Donaldson: On the percentage of Water in the Brain and in the Spinal Cord of the Albino Rat, *Journ. of Neurol. and Psychol.*, xx, p. 143, 1910.

<sup>6</sup> *Ibid.*



phosphatides, and cholesterol; in other words, an increase in substances which predominate in the fibres (medullated sheath) during growth."<sup>7</sup> These same differences are to be found in all

TABLE I.

*Relative proportion of the proximate constituents of the brain of the albino rat at birth and when adult.*

	ALBINO RAT (AT BIRTH)		ALBINO RAT (ADULT)
Moist weight of one brain.....	0.25	0.25	1.667
Solids in per cent.....	10.42	10.42	21.9
Dry weight of one brain.....	0.026	0.026	0.380
Number of brains in sample.....	100	100	31

*In relative proportions of solids.*

Proteins.....	57.16	57.30	48.5
Phosphatides.....	14.8	15.6	22.0
Cerebrosides.....	0.0*	0.0	9.0
Sulphatides.....	1.5	1.4	4.6
Organic extracts } .....	16.5	19.3	9.8
Inorganic const. }			
Cholesterol } i.....	(10.04)	(6.4)	(6.1)
Undetermined }			
Total S.....	0.96	1.04	0.58
Total P.....	1.82	1.92	1.39

*Distribution of sulphur in per cent of total S.*

Protein S.....	31.02	30.02	64.2
Lipoid S.....	3.2	2.8	15.6
Neutral S.....	49.14	47.26	14.2
Inorganic S.....	16.6	19.95	6.0

*Distribution of phosphorus in per cent of total P.*

Protein P.....	13.3		6.8
Lipoid P.....	33.2	33.0	67.6
Water-soluble P.....	53.5	53.6	25.6

\* Mendel, L: *Amer. Journ. of Physiol.*, xxi, p. 104, 1908.

† By difference.

<sup>7</sup> W. Koch and S. A. Mann: A Comparison of the Chemical Composition of Three Human Brains at Different Ages, *Journ. of Physiol.*, xxxvi, pp. 1-3. (From the Proceedings of the Physiological Society, November 23, 1907).



TABLE II.

*Relative proportions of the proximate constituents of the brain of the fetal pig at different ages.*

Year of analysis.....	50 MM. PIG FETUS			100 MM. PIG FETUS			200 MM. PIG FETUS
	'11	'12	'12	'11	'12	'12	'11
Moist weight of one brain.....	0.40	0.43	0.47	1.8	1.91	2.15	10.1
Solids in per cent.....	8.75	9.87	9.04	9.1	8.98	8.98	
Dry weight of one brain	0.035	0.042	0.042	0.164	0.171	0.193	
Number of brains in sample.....	65	111	109	35	27	27	

*In relative proportions of solids.*

Proteins.....	56.6	58.2	54.61	51.5	51.81	52.34	43.8
Phosphatides.....	13.0	15.04	15.79	15.7	16.31	14.85	17.2
Cerebrosides.....							
Sulphatides.....	2.4?	0.8	1.05	1.8?	0.96	0.84	0.00?
Organic extract	} 22.20	20.5	23.84	24.2	24.92	25.44	23.2
Inorganic const.							
Cholesterol.....	2.4*	2.4*	2.4*	4.4*	4.4*	4.4*	
Undetermined†.....	(3.4)	(3.06)	(2.31)	(2.4)	(1.6)	(2.13)	(8.42)
Total S.....	0.67	0.59	0.58	0.59	0.57	0.55	0.55
Total P.....	1.74	1.85	1.90	1.76	1.91	1.82	1.45

*Distribution of sulphur in per cent of total S.*

Protein S.....	54.3	57.3	55.8	58.4	57.8	55.66	60.9
Lipoid S.....	7.2?	2.67	3.59	6.1?	3.36	2.98	0.00?
Neutral S.....	29.3	29.4	27.6	26.7	28.97	31.68	25.5
Inorganic S.....	9.0	10.67	13.0?	9.0	9.93	9.6	13.33

*Distribution of phosphorus in per cent of total P.*

Protein P.....		15.7	14.0		14.5	14.6	5.2
Lipoid P.....	31.6	32.4	29.6	35.5	34.5	31.6	46.3
Water-soluble.....	53.6	51.8	56.2	.	50.9	53.8	48.5

\* Mendel, L.: *Amer. Journ. of Physiol.*, xxi, p. 103, 1908.

† By difference.

(?) Indicates doubtful result.

TABLE III.

*Relative proportions of the proximate constituents of the brain of the fetal pig at different ages compared with the brain of the albino rat at birth. (Averages of the foregoing determinations.)*

	PIG FETUS		ALBINO RAT	
	50 mm.	100 mm.	at birth	adult
Moist weight of one brain.....	0.433	1.90	0.25	1.667
Solids in per cent.....	9.22	8.99	10.42	21.9
Dry weight of one brain.....	0.039	0.171	0.026	0.380
Number of brains in sample.....	95	30	100	31

*In relative proportions of solids.*

Proteins.....	56.47	51.88	57.23	48.5
Phosphatides.....	15.41	15.62	15.2	22.0
Cerebrosides*.....	0.0	0.0	0.0	9.0
Sulphatides.....	0.92	0.90	1.45	4.6
Organic extract } Inorganic const. }	22.18	24.69	17.9	9.8
Cholesterol.....	2.4†	4.4†		
Undetermined‡.....	(2.59)	20.49	(8.22)	(6.1)
Total S.....	0.585	0.57	1.00	0.58
Total P.....	1.83	1.83	1.87	1.39

*Distribution of sulphur in per cent of total S.*

Protein S.....	55.8	57.28	30.52	64.2
Lipoid S.....	3.13	3.17	3.0	15.6
Neutral S.....	28.7	29.11	48.2	14.2
Inorganic S.....	9.83	9.51	18.27	6.0

*Distribution of phosphorus in per cent of total.*

Protein P.....	14.8	14.55	13.3	6.8
Lipoid P.....	31.2	33.8	33.1	67.6
Water-soluble P.....	53.8	52.3	53.55	25.6

\* Cerebrosides not determined in fetal brains. Not present according to Mendel.

† Mendel, L: *Amer. Journ. of Physiol.*, xxi, p. 103, 1908.

‡ By difference.

nervous tissue during growth and may therefore be used in making a comparison between the brain of the new born rat and that of the fetal pig to determine which is the more immature.

We may now proceed to consider in detail the comparison of the various constituents<sup>8</sup> in the brain of the new born rat and the pig fetus.

*Water.* The per cent of water in the brain of the new born rat is closely similar to, but a little lower than, that of either the 50 mm. or 100 mm. pig fetus. This would indicate that the rat is of about the same physiological age as these fetuses, since the differences are within the limits of error.

*Protein.* The per cent of protein in the total solids is higher in the brain of the new born rat than in either that of the 50 or the 100 mm. pig fetuses. Since the per cent of protein is highest in the youngest material, this is an indication that the rat's brain is less mature than that of the 100 mm. pig fetus, but not very different from the 50 mm. fetus.

*Phosphatides.* The per cent of phosphatides is the same in the new born rat as it is in the 50 and the 100 mm. pig fetus. This would indicate a close agreement in physiological age between these two. This is the lowest phosphatide content yet obtained in an analysis of the brain tissue and approaches that observed in the suprarenal, which, among all the organs, comes closest to that of nervous tissue in chemical composition.

*Cerebrosides.* These are absent in both the new born rat, and in the pig fetus, as is to be expected in nervous tissue before medullation.

*Sulphatides.* The percentage of sulphatides is about the same in the new born rat as in the pig fetus, which indicates the same age.

*Organic extractives and inorganic constituents.* These are somewhat higher in the pig fetus than in the new born rat and, except as this is associated with the greater per cent of lymph in the embryonic material, it would indicate it to be more immature than the new born rat.

<sup>8</sup> The nature and significance of the constituents will be discussed in the third paper of this series.



*Cholesterol.* The figures for cholesterol were not determined by me, but were taken from Mendel<sup>9</sup> and incorporated here for the sake of completeness.

This leaves *undetermined* from 2 to 3 per cent which is not more than would be expected in the errors involved in making so many determinations from one tissue and calculating approximate constituents from assumed factors.

*The distribution of sulphur* in per cent of total sulphur is widely different in the two forms, but as this is not correlated with age but is apparently a species peculiarity, the results are not out of harmony with the foregoing conclusions.

*The distribution of phosphorus* between the protein, lipid and water-soluble phosphorus is closely similar in the rat and the 50 and 100 mm. pig fetus, showing the physiological ages to correspond.

The remarkably high figure for neutral and inorganic sulphur in the rat at birth requires an explanation but it is not possible to give this with the data so far at hand.

The general conclusions from these figures are, that from a chemical standpoint the brain of the new born rat is about as immature as that of the 100 mm. pig fetus, being on the whole a little less differentiated than the latter.

The differences between the brain of the 50 mm. and the 100 mm. pig fetus are not marked, and this would indicate that there occurs between these ages an increase in weight unaccompanied by any significant change in chemical composition. This would correspond with the results of Mendel<sup>10</sup> and Raske<sup>11</sup> who found that in the brains from these young fetuses there is no chemical distinction between grey and white matter. Moreover the brain of the 50 mm. pig fetus is the youngest which it is practicable to obtain for analysis and even at this age the tissues are so watery and filled with lymph that some error is thereby introduced in the analysis of the constituent tissues.

Since the brain of the 50 mm. pig fetus shows no material differences from that of the 100 mm. pig fetus and the latter is no more

<sup>9</sup> L. B. Mendel and Charles S. Leavenworth: Chemical Studies on Growth. IX. Notes on the Composition of Embryonic Muscular and Nervous Tissues, *Amer. Journ. of Physiol.*, xxi, p. 103, 1908.

<sup>10</sup> *Ibid.*

<sup>11</sup> Raske: *Zeitschr. f. physiol. Chem.*, x, p. 340, 1886.

immature chemically than that of the new born rat, it appears that the new born rat's brain is as young nervous material as can conveniently be analyzed at present: and it forms, therefore, a convenient starting point for the study of the chemical differentiation of the central nervous system during growth.

CHEMICAL RESULTS CONFIRMED BY PHYSIOLOGICAL AND ANATOMICAL DATA.

It is astonishing that chemically the brain of the new born rat should be as immature as that of the 100 mm. pig fetus, but, surprising as this fact is, it is substantiated by a comparison of the structure of the cerebellum of these two animals and of their behavior at the time of birth.

It is a well-known fact that the rat is born in a very immature state, with its eyes shut, and when first born, is capable only of movements involved in sucking, bending the body and tail and making a squeaking noise.<sup>12</sup> The pig, on the other hand, "is born with its eyes open and requires no assistance as a rule in making its start in life. It is more or less able to walk around as soon as born."<sup>13</sup> Such a state of activity in the rat is not reached until the period of weaning 17-21 days after birth.

This difference in physiological behavior is correlated with the relative development of the cerebellum of the two animals; particularly as indicated by the development and transformation of the outer granular layer of cells. A comparison of this layer in both animals, founded on the observations of Addison<sup>14</sup> who studied the different layers of the cerebellum in the albino rat, and of Takasu<sup>15</sup> who studied these same layers in the pig fetus, brought out the following facts:

<sup>12</sup> Wm. H. F. Addison: The Development of the Purkinje Cells and the Cortical Layers in the Cerebellum of the Albino Rat, *Journ. of Comp. Neurol.*, xxi, p. 476, 1911.

<sup>13</sup> Forbes: personal communication.

<sup>14</sup> Wm. H. F. Addison: *Journ. of Comp. Neurol.*, xxi, p. 464, 1911.

<sup>15</sup> K. Takasu: Zur Entwicklung der Ganglienzellen der Kleinhirnrinde des Schweines, *Anat. Anz.*, xxvi, pp. 225-32, 1905.



	RAT	PIG
First appearance of cells in outer granular layer.....	19-day fetus	50 mm. fetus
Division of layer into two zones inner and outer.....	At birth	100 mm. fetus
Disappearance of cells from inner zone of layer.....	21 days after birth	300 mm. fetus

The first appearance of the cells in the outer granular layer of the cerebellum in the rat is in the 19-day fetus, and in the pig in the 50 mm. pig fetus. At this time the cells are settled in a thin layer (two rows deep) around the outer edge of the cerebellar cortex. This layer increases until a considerable depth is filled in with cells which soon separate into two strata, an outer and an inner; this separation takes place in the rat at birth, and in the pig fetus when it is 100 mm. in length. The cells from the outer granular layer now begin to migrate to the inner granular layer and the disappearance of the cells from this outer granular layer, which corresponds with the time of securing motor control in an animal, occurs in the rat at the twenty-first day of life, and in the pig when this is from 200 to 300 mm. in length, or at birth. These facts show, therefore, that the new born rat is as developed with respect to motor activity as the 100 mm. pig fetus, and that the rat at weaning (17-21 days after birth) and the pig at birth are at corresponding physiological ages. The conclusion from this anatomical comparison, namely, that the 100 mm. pig fetus and the rat at birth are of like physiological age, fully confirms, therefore, the conclusion drawn from both chemical and physiological evidence already adduced.

We now ask the question, how far this result, that the nervous system of the new-born rat is chemically as old as that of the 100 mm. pig fetus, agrees with observations made by Donaldson, that the rate of growth and percentage of water in the mammalian nervous system (represented by the brains of man and the rat) agree in the two forms at equivalent ages, thus indicating that the nervous systems are in corresponding physiological states at equal fractions of the life cycles.<sup>16</sup>

<sup>16</sup> H. H. Donaldson: A Comparison of the White Rat with Man in respect to the Growth of the Entire Body, *Boas Anniversary Volume*, 1906, pp. 5-26.



It remains therefore to inquire whether the chemical and behavior relations between the rat and pig which have just been pointed out, occur at equivalent ages in these two forms.

Great difficulty was experienced in finding any statements concerning the age of the pig fetuses. The statements of different authors did not always agree, but the two which agreed closest were those of Bradley<sup>17</sup> and Coe.<sup>18</sup> Bradley compared the length of the embryos with the time from coition; Coe estimated the age from the rate of development of embryos of other mammals. While considerable uncertainty thus attaches itself to these figures<sup>19</sup> it may be assumed that the 50 mm. pig fetus is about 40 days old from conception: the 100 mm. fetus is 55–62 days; and the 200 mm. fetus is from 88–90 days from conception.

We find in the rat the period of gestation is 21 days and its span of life three years (Donaldson), or a total age of 1116 days; in the pig the period of gestation is 125 days and its normal span of life, as far as could be ascertained, is 20 years,<sup>20</sup> or 7425 days. The rat, therefore, lives about one-sixth as long as the pig. Assuming that the rat at birth has lived  $\frac{21}{1116}$ , or  $\frac{1}{53}$ , of its total life, the 60-day pig fetus will have lived  $\frac{60}{7425}$ , or  $\frac{1}{123.75}$ , of its life. It appears then, if the total length of life given is correct for both animals and the numbers used for the divisors in each case are really comparable as they stand, that we do not have corresponding physiological conditions of the brain at equivalent ages, for these brains

<sup>17</sup> O. C. Bradley: On the Development of the Hind Brain of the Pig, *Journ. of Anat. and Physiol.*, xl, Part I, p. 1.

<sup>18</sup> Mendel refers to Professor Coe in Chemical Studies on Growth. I. The Inverting Enzymes of the Alimentary Tract, especially in the Embryo, *Amer. Journ. of Physiol.*, xx, p. 90, 1907–1908.

<sup>19</sup> Bradley makes the statement, that "although the age of the different embryos is given, it is not intended that it should signify more than the time which elapsed between the time of coition and the time when the mother was destroyed. . . . In embryos taken from two litters it not infrequently happens that those which should be further advanced in development, judging from the period which has elapsed since sexual congress took place, are as backward, or even more backward, than those of the 'younger' litter." A more definite way to determine the age of an embryo would be, according to Mall, by ossification. No data were available however for a comparison between the rat and the pig.

<sup>20</sup> Longevity, *Encyclopædia Britannica* (Eleventh Edition), xvi, p. 975.

are found to be in corresponding states at the  $\frac{1}{3}$  and the  $\frac{1}{10}$  part of the total life cycles. Had the relation, in the form stated above, held, these fractions should have been identical. It is only fair to add, however, that in view of the absence of precise information concerning the pig and in view of the fact that the early days of gestation are used for cell division accompanied by only slight differentiation, too much stress should not be laid on the relation here given.

On the other hand, instead of taking the end of life as the fixed point of our calculations, we may consider the time when motor control is obtained to indicate closely corresponding states of the central nervous system. In the rat, motor control is obtained at 42 days from conception, and in the pig at 125 days, that is, at birth. If the law of corresponding states is correct, the nervous system of these two animals should be in corresponding conditions at the same fractions, either  $\frac{1}{4}$ ,  $\frac{1}{2}$ , or  $\frac{3}{4}$  of these periods. This is found to be the case, for the rat is born after 21 days' gestation. This would be just half way between the two fixed points of conception and time of gaining motor control and this corresponds in the pig to just one-half of its gestation period or about 62 days, which is the age of the 100 mm. pig.

It was actually found, both chemically and anatomically, that the nervous systems of these two animals were in the same state of development at these respective periods and it appears from these observations that Donaldson's law may hold, if put in the form: the nervous systems of mammals are in the same physiological state at equal fractions of their total periods of development.

In conclusion it gives me great pleasure to thank Dr. H. H. Donaldson and Prof. A. P. Mathews for their many suggestions in connection with this problem and for their aid in getting this paper ready for publication. The problem itself was suggested to me by my brother and forms the first of a series of papers which are to follow from time to time, on the chemical differentiation of the central nervous system, and on which he was engaged at the time of his death. The work was carried out in the Laboratory of Biochemistry and Pharmacology of the University of Chicago and was aided by a grant from the Wistar Institute of Anatomy and Biology, Philadelphia.



## SUMMARY.

1. A quantitative determination of the constituents of the brain of the albino rat at birth shows it to be chemically as undifferentiated as the brain of a 50 mm. or 100 mm. pig fetus.

2. There is little difference in chemical composition between the 50 mm. and the 100 mm. pig fetus brain.

3. Since the 50 mm. fetus brain is the youngest which can be analyzed and this closely resembles the 100 mm. fetus, and this in turn is no more immature than the new born rat, it appears that the brain of the new born rat is sufficiently immature to serve as a starting point in a study of the chemical differentiation of the brain during growth.

4. That the brain of the new born rat is as immature as the 100 mm. pig embryo is shown, also, by the similarity of the changes in the outer layer of cells of the cerebellar cortex in both animals previous to gaining motor control, and by the animal's behavior at this period.

5. If the nervous systems are assumed to be in corresponding states when motor control is obtained, and Donaldson's law is correct, that the nervous system is in the same state at corresponding physiological ages, then the brain of the rat at birth should correspond chemically with the 100 mm. pig fetus brain. This is found to be the case.





## CONTRIBUTIONS TO THE CHEMICAL DIFFERENTIATION OF THE CENTRAL NERVOUS SYSTEM.

### II. A COMPARISON OF TWO METHODS OF PRESERVING NERVE TISSUE FOR SUBSEQUENT CHEMICAL EXAMINATION.

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(Received for publication, February 20, 1913.)

With valuable biological material it is sometimes desirable to make water estimations and the estimations of the other constituents on the same sample. This can be done somewhat indirectly if the method already described<sup>1</sup> of placing the fresh, weighed tissues immediately in 95 per cent alcohol is used.

To see whether tissues in which the water had been determined by drying could thereafter be analyzed by the methods referred to above and would yield the same proportion of the various constituents as these same tissues treated by the alcohol method, an analysis was made of the brains and spinal cords of albino rats which had been dehydrated in these two ways. The possible drawbacks of the heat method of determining moisture, namely, the oxidation or decomposition of part of the material and the evaporation of volatile constituents, are obvious, but we had no definite knowledge of how serious the errors involved in the method might be in practice.

To determine to what extent these changes took place and what they were, we analyzed material which had been dried at 95°C. for one week and which at the end of this time had been placed in alcohol, and compared it with similar material which had been placed directly in alcohol. The results are given in the table.

It may be seen by a comparison of the results of the two analyses in the table, that decompositions seriously affecting the analyses are produced by heat drying, particularly in the case of the brain. The differences are most marked in the phosphorus compounds.

<sup>1</sup> Koch, W.: *Journ. Amer. Chem. Soc.*, xxxi, pp. 1353-4, 1909.



*Comparison of brains and cords dried at 95°C. with brains and cords placed directly in alcohol without heating.*

	ENCEPHALON		CORDS	
	Direct into alcohol	Dried at 95°C.	Direct into alcohol	Dried at 95°C.
Laboratory number.....	W 13	W 18	W 9	W 20

*In per cent of total solids.*

Proteins.....	48.5	47.9	32.8	29.6
Phosphatides.....	22.0	16.2	25.3	22.1
Cerebrosides.....	8.4	(?)	12.5	14.4
Sulphatides.....	4.5	4.6	7.0	6.7
Organic ext. } .....	9.8	12.4	7.6	8.0
Inorganic const. }				
Undetermined lipoids.....	6.8*		14.8*	19.2*
Total S.....	0.58	0.59	0.45	0.42
Total P.....	1.39	1.31	1.44	1.42

*Distribution of sulphur in per cent of total S.*

Protein S.....	63.8	64.6	53.7	53.3
Lipoid S.....	15.6	15.6	30.9	32.1
Neutral S.....	14.5	14.0	10.3	9.5
Inorganic S.....	6.1	6.0	5.0	5.0

*Distribution of phosphorus in per cent of total P.*

Protein P.....	6.8	8.1	5.6	5.0
Lipoid P.....	67.6	55.1	77.4	69.2
Water Sol. P.....	25.6	36.8	17.0	25.8

\* Obtained by difference.

By drying there has been a destruction of the phosphatides involving a change in the distribution of phosphorus in per cent of total phosphorus; that is, a considerable amount of lipid phosphorus is changed to water-soluble phosphorus. There was no change in the sulphur distribution. It will be noticed that the phosphatides of the cord are more resistant to heat than those of the brain, a point of sufficient interest to justify repetition.

We conclude, then, that the determination of water by drying at 95°C. cannot safely be used, if it is desired to determine in the same sample the relative proportions of the solid constituents; and that the indirect method already described is far superior for this purpose.



## CONTRIBUTIONS TO THE CHEMICAL DIFFERENTIATION OF THE CENTRAL NERVOUS SYSTEM.

### III. THE CHEMICAL DIFFERENTIATION OF THE BRAIN OF THE ALBINO RAT DURING GROWTH.

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(Received for publication, July 1, 1913.)

#### INTRODUCTORY STATEMENT.<sup>1</sup>

The transformations which occur in the brain during growth offer a particularly enticing field for the study of chemical differentiation, not alone because of the very great interest attaching to the solution of the problem of the chemical basis of its functions, but because its structural differentiation during growth is very marked. On the one hand there is the formation of a large amount of new material composing the medullary sheath of the nerve fibers, and, on the other hand, the appearance of a quantity of a peculiar supporting tissue, the neuroglia. The chemical changes during growth should, therefore, be very marked; and it is of interest to discover how far our chemical methods enable us to follow such obvious structural modifications.

<sup>1</sup> Waldemar Koch died at Chicago February 1, 1912. As Associate in Biological Chemistry at the Wistar Institute of Anatomy and Biology, he spent the autumn of 1910 and of 1911 in Philadelphia working mainly on matters connected with this research. This paper has been prepared in considerable part from results of analyses made by me under the direction of my brother, and from a manuscript written by him. Many additional analyses, which he had planned, have been made and incorporated into the series. The interpretations of the results have been left to a large extent, in his words. I have been assisted in its preparation for publication by Professor A. P. Mathews and Dr. H. H. Donaldson, the aid of both of whom I gratefully acknowledge.—M. L. KOCH.

The selection of chemical methods for such a study was largely guided by the principle now coming to be generally accepted, namely, that in living matter we are not dealing with an aggregation of more or less similar, highly organized and necessarily complex molecules (Riesenmolekül of Pflüger), but rather, with a more or less heterogeneous substratum in which dissimilar and not necessarily highly complex molecules, or their dissociated particles, are engaged in a series of correlated chemical reactions. The larger aggregates may be conceived as either not taking part directly in chemical activity, or as helping in the control and localization of the chemical reactions, just as in a photographic dry plate, the presence of the gelatin makes possible a high degree of localization of the photo-chemical reaction. We aimed, therefore, to stop the chemical activities at definite given stages during the growth period and then to observe the differences which could be demonstrated. From such data we then drew conclusions as to the nature of the transformations which had occurred in the interval.

The methods of collecting the material were devised with this end in view, namely, to stop all chemical activity as rapidly and completely as possible. The sources of error due to post mortem changes then became constant, and we are in reality following a principle that has long been in use in histological studies. For the preserving agent, alcohol was selected, as it is the least apt to interfere with the further chemical procedure, and, in fact, treatment with alcohol represents a step in the process.

In the selection of the chemical methods for this series two points were kept in mind:

1. The necessity of correlating the chemical observations with the known facts of structure, to the interpretation of which they should add a greater precision. As an example of this, there were studied the sulphatides (lipoid sulphur) which are intimately associated in the nervous system with the sheaths of the medullated nerve fibers.

2. The collection of data, which, correlated with function, would give the physiologist a better knowledge of the nature of his material and thus enable him to do more than speculate as to the probable nature of the processes involved in the phenomena

he is observing. As an example of this, there was studied the ratio between neutral sulphur and protein sulphur, a ratio which correlates closely with the decrease in metabolic activity associated with the growth of the nervous system from birth to maturity.

The general plan of the chemical technique has been first to block out the material into larger groups of substances and then carry the procedure of separation into greater detail. The necessity of working with data which represent something definite from the point of view of the chemist, has also been kept in mind.

The following outline illustrates the extent to which the chemical procedure has been carried up to the present.

*Outline illustrating the separation of constituents by the method employed,<sup>2</sup> classified according to their state of aggregation.*

	ENCEPHALON DIVIDED BY STATE OF AGGREGATION INTO:			
	COLLOIDAL (FRACTION 1 AND 4)		NON-COLLOIDAL (FRACTION 2 AND 3)	
	Proteins (Fract. 4)	Lipoids (Fract. 1)	Organic Extractives (Fract. 2 and 3)	Inorganic Constituents (Fract. 2 and 3)
Proximate constituents	(Include supporting structures)	Phosphatides Cerebrosides Sulphatides { Cholesterol Undetermined		Sodium Potassium Calcium Magnesium Chlorides
Sulphur combinations	Protein S	Lipoid S	Neutral S	Inorganic S (sulphates)
Phosphorus combinations	Protein P	Lipoid P	Organic extractives P	Inorganic P (phosphates)

For an explanation of the chemical procedure followed for this separation the following outline has been inserted.

<sup>2</sup> The method employed for this separation is described in an earlier paper by W. Koch and coworkers: *Journ. of the Amer. Chem. Soc.*, xxxi, pp. 1342-1361, 1909.



*Moist Brain Tissue: Add alcohol and extract alternately with alcohol and ether.<sup>3</sup>*

EXTRACT (FRACTION 1 AND 2)		RESIDUE (FRACTION 3 AND 4)	
EVAPORATE TO DRYNESS, EMULSIFY WITH WATER, PPT. WITH $\text{CHCl}_3$ IN 0.5 PER CENT $\text{HCl}$ SOLUTION		DRY, WEIGH AND EXTRACT WITH HOT WATER	
Ppt. (Fract. 1):	Filtrate (Fract. 2):	Filtrate (Fract. 3):	Residue (Fract. 4):
Lipoids	Organic extractives Inorganic constituents	Organic extractives Inorganic constituents	Proteins

Organic extractives in Fraction 2 and 3 are equal to total organic extractives.

Inorganic constituents in Fraction 2 and 3 are equal to total inorganic constituents.

Fraction 1 and 2 are soluble in alcohol (85-95 per cent).

Fraction 3 is insoluble in alcohol; soluble in hot water.

Fraction 4 is insoluble in alcohol and hot water.

For a clearer understanding of the terms used in this series of papers, the following interpretation of the *chemical nature, anatomical distribution, and physiological significance* of the substances determined, with special reference to the nervous system based both on the studies already made and those presented in this paper, is given below.

### *Proteins.*

*Chemistry.* These represent complex combinations of amino-acids rendered insoluble in water by coagulation with hot alcohol. This fraction has been exhaustively extracted with hot alcohol and should retain only traces of lipoids and fats. The nucleoproteins and the neurokeratin are included in this fraction.

*Anatomical distribution.* In the part of the nervous system rich in cells (cortex) the proportion of the proteins is larger than in the white matter. Some of the nucleoproteins are supposed to be associated with the chromatin and Nissl substance of the nerve cell. The remainder of the nucleoproteins are represented by the nuclei of the glia cells scattered through-

<sup>3</sup> Although ether is used in the extraction following the first alcohol, it does not remove any considerable amount of material and need not be considered in the above scheme.

out the nervous system. Neurokeratin occurs in the medullated sheath of the nerve fiber. The other proteins occur in the axon of the nerve fiber as well as in the cell body and its dendrites.

*Physiological significance.* The proteins have usually been considered as the essentially living part of the protoplasm, but some of them, like neurokeratin, are undoubtedly inactive and represent supporting structures. The same may be said of the proteins which make up the fibers of the glia cells. It is therefore impossible to tell at the present time to just what extent and in what proportion the proteins are involved in the chemical activities of the nervous system. The significance of the neutral sulphur compounds, which represent simpler cleavage products of the larger protein aggregates, will be discussed later as having an important bearing on this point (see p. 431).

#### *Phosphatides.*

*Chemistry.* These represent complex combinations of fatty acids, phosphoric acid, glycerin, and nitrogen complexes of the nature of choline, and include among other things lecithin and kephalin. The chemistry of this group is very much in need of revision, as some of its members are not so simple as the older work of Hoppe-Seyler has led us to infer. The group does not include lecithin in combination with sulphur or cerebrin. The phosphatides as here given are calculated from the phosphorus of the lipid fraction on the assumption that they have an average molecular weight of 800. Correction must be made for the phosphorus of the sulphatides.<sup>4</sup>

*Anatomical distribution.* Comparison of cortex and corpus callosum<sup>5</sup> indicates that the phosphatides are not very differently distributed between cell body and nerve fiber. Analyses of the brain at a period when medullation has not begun, but when the cell processes are growing freely, indicate that the phosphatides are largely associated with the axon. If mitochondria consist largely of phosphatides, as has been suggested, the observations of Cowdry would give us a picture of their distribution in the cell body. The absence of mitochondria in the axon, which is known to contain phosphatides, would not argue against this, as there is some evidence that the phosphatides of the processes and the cell body are different in their behavior.

*Physiological significance.* The phosphatides, like the proteins, may be considered to be intimately associated with the vital processes of the living protoplasm. Their colloidal nature and relation to inorganic ions, as well

<sup>4</sup> *Calculations for phosphatides.* The total lipid phosphorus found times 25.77 gives the phosphatides, on the basis that 3.88 per cent of the phosphatides consist of phosphorus. Since 51.2 per cent of the sulphatides are phosphatides, that amount was deducted from the total phosphatides found. The difference was considered as free phosphatides.

<sup>5</sup> Koch, W.: *Amer. Journ. of Physiol.*, xi, pp. 326-328, 1904.



as their instability towards heat,<sup>6</sup> lend support to this idea. They probably occur largely in the cytoplasm, cell body and its branches, where they may act as oxygen carriers, as has been suggested by the work of Koch and Mostrom.<sup>7</sup>

#### *Cerebrosides.*

*Chemistry.* Complex combinations of fatty acids, galactose, and possibly other hexoses with a nitrogen complex of the nature of sphingosine. The cerebrosides are calculated from the lipid sugar on the assumption that they yield on hydrolysis 21.8 per cent of reducing sugar, the amount found by Thierfelder in his cerebrin. Correction must be made for the cerebrin content of the sulphatides.<sup>8</sup>

*Anatomical distribution.* Although the cerebrosides are occasionally met with in other tissues, they occur in largest amount in the medullated nerve fiber, and their quantity increases as medullation proceeds. The rather large amount found in the cortex<sup>9</sup> on chemical analysis indicates that they may predominate in the fibers of that region.

*Physiological significance.* As laid down in the medullated nerve fiber, the cerebrosides most probably serve only a mechanical function and are not available as sources of energy in spite of their carbohydrate and fatty acid content.

#### *Sulphatides.*

*Chemistry.* These represent the combination of a phosphatide with a cerebroside by means of a sulphuric acid group in ester combination.<sup>10</sup> The sulphatides are estimated from the lipid sulphur on the basis of a sulphur content of 2 per cent, based on the analysis of a purified compound.<sup>11</sup>

<sup>6</sup> Koch, W and Koch, M. L.: this *Journal*, xiv, pp. 281-282, 1913.

<sup>7</sup> Koch, W and Mostrom, H. T.: *Journ. of Pharm. and Exp. Ther.*, ii, No. 3, p. 265, 1910.

<sup>8</sup> *Calculations for cerebrosides.* The cerebrosides, from the lipid fraction, on hydrolysis for twenty-four hours with a weak solution of HCl (75 cc. of water containing 3 cc. concentrated HCl), yield 21.8 per cent by weight galactose. The calculations for cerebrosides were made on the assumption that galactose and glucose were equivalent in reducing power and the weight of galactose was thus determined from Munson and Walker's tables for glucose. (*Journ. Amer. Chem. Soc.*, xxviii, p. 663). The corrected weight of total galactose to cerebrosides was then made on the basis that 21.8 per cent of the latter is galactose. Finally since 42.9 per cent of the sulphatides consist of cerebrosides this amount was deducted from the total cerebrosides found. The difference was considered as cerebrosides.

<sup>9</sup> Koch, W. and Mann, S. A.: *Archives of Neurology and Psychiatry*, iv, p. 33, 1909.

<sup>10</sup> Koch, W.: *Zeitschr. f. physiol. Chem.*, lxx, p. 94, 1910.

<sup>11</sup> *Calculations for sulphatides.* These are considered to be of the general formula:



If it were desirable to recognize the chemical identity of the much abused protagon, the sulphatides might be considered as purified products. Protagon could be much more safely calculated from the lipoid sulphur than from the lipoid sugar as Noll<sup>12</sup> has attempted. The sulphur content of protagon preparations, when it has not been simply ignored, is variously reported as 0.5 and 1.0 per cent.

*Anatomical distribution.* The sulphatides, like the cerebrosides, increase parallel with the growth of the medullary sheath and may be considered as essential constituents of that structure. The fact that the sulphatides, as the result of more recent work, have been found to be pretty generally distributed in other tissues, indicates that they might occur in the cell body of the neurone, although a comparison of the analyses of cortex and corpus callosum does not make this very probable. The sulphatides have an important function in the maturing of the nerve fiber and give the Weigert staining reaction in a very characteristic manner.

*Physiological significance.* Their colloidal nature and the peculiar combination into which the sulphatides enter with potassium, suggests that they may have an important relation to the nerve impulse and to the phenomena of conductivity in general.

#### *Organic extractives and inorganic constituents.*

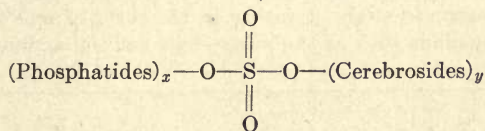
*Chemistry.* This group represents essentially the water-soluble, non-colloidal constituents of the nervous system. The older method of estimating the inorganic constituents by the ash has been abandoned as too inaccurate. The principal reason for reporting the above group is to give an idea of the ratio between the colloidal and non-colloidal constituents.

*Anatomical distribution.* The group occurs in large quantity in the cell body, although some is also present in the axon of the nerve fiber.

*Physiological significance.* This group is a rough index of the amount of metabolic activity going on in the tissue, as it represents at the same time the end products of chemical activity, as well as the culture media from which the more complex combinations are built up.

#### *Undetermined (cholesterol).*

This fraction is represented in the nervous system to a certain extent by cholesterol, which has not been directly estimated. Besides this, how-



containing 2.0 per cent sulphur, 42.9 per cent cerebrosides, and 51.2 per cent phosphatides. Then we have,

$$\frac{(\text{Lipoid sulphur} \times 50)}{\text{weight of dry substance}} = \text{per cent of sulphatides in dry substance.}$$

<sup>12</sup> Noll, A.: *Zeitschr. f. physiol. Chem.*, xxvii, p. 370, 1899.

ever, all the errors of analysis, as well as of such calculations as are based on assumed factors, enter into this fraction. After accounting for the cholesterol in the brain of the 50 and 100 mm. pig fetus,<sup>13</sup> in which this was estimated directly by Mendel, there remained undetermined 2 to 3 per cent of the total solids. Considering the number of groups estimated, this is not a very discouraging result. (In other tissues, which contain little cholesterol, the undetermined is recorded as neutral fat.)

*Anatomical distribution.* Cholesterol is principally of interest as a constituent of the medullary sheath to which it adds a sort of mechanical stability. But it is present in the cell bodies also, possibly contributing to the cell membranes. According to Lorrain Smith<sup>14</sup> it is one of the substances responsible for the color which the medullary sheath gives with Weigert's stain.

*Total sulphur and total phosphorus.*

It may not be out of place to state briefly the reasons for selecting these two elements for special determination in preference to others. As far as the phosphorus is concerned, the importance of the nucleins to all living cells and the phosphatides to the nervous system in particular, amply justify its selection. The reason for selecting sulphur in preference to the much more generally studied nitrogen, may, however need a word of explanation.

Nitrogen is studied for two reasons: because it is an important element in the building up of the proteins, and because it is easy of estimation.

Sulphur is just as characteristic of proteins, in fact more so, as it does not enter into the non-protein groups such as the nucleic acids. Among the lipoids, too, sulphur enters into only one group, the sulphatides, while nitrogen occurs in all except cholesterol.

In other words, to estimate sulphur in the protein fraction is to estimate an element essentially characteristic of the more truly protein part. To estimate it in the lipid fraction, enables one to distinguish one particular, and, as growth curves show, a very interesting group of lipoids. Besides, as has already been pointed out in a previous paper<sup>15</sup> sulphur occurs in the tissues in several states of oxidation and thus gives us some indication of the intensity of reactions of oxidation which are so important to growing tissues, and about which we know so little. It seems wise therefore to estimate the sulphur, and in case there are any special reasons to study nitrogen, to study it rather in the form of one of its definite groups of compounds such as the purine bases or the amino-acids.

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<sup>13</sup> Koch, Mathilde L.: this *Journal*, xiv, pp. 267-279, 1913.

<sup>14</sup> Smith, Lorrain: *Journ. of Path. and Bact.*, xv, pp. 179-181, 1911.

<sup>15</sup> Koch, W. and Upson, F. W.: *Proc. Soc. for Exp. Biol. and Med.*, vii, pp. 5-6, 1909.



*Distribution of sulphur.*

*Chemistry.* **PROTEIN S.** This group represents sulphur in various amino-acid combinations such as cystine or cysteine. The proteins in which this sulphur fraction is found have been coagulated and rendered insoluble in water by treatment with hot alcohol.

**LIPOID S.** Ethereal sulphuric acid combinations are discussed under sulphatides.

**NEUTRAL S.** This group of compounds represents the total non-colloidal, water-soluble combinations of sulphur, minus the inorganic sulphates. As far as studied, they resemble in all their reactions a similar group found in the urine and called by Bondzynski proteinic acids. They represent, probably, larger cleavage products of the protein molecule or complex non-coagulable, water-soluble polypeptides somewhat altered by processes of oxidation. The sulphur of this fraction is represented essentially by compounds included among the organic extractives, and the sulphur is most often in an oxidized form like taurine or ethereal sulphate.

**INORGANIC S** (inorganic sulphates). Derivatives of sulphur directly precipitated by barium chloride in hydrochloric acid solution.

*Anatomical distribution and physiological significance.* The **LIPOID S**, as has already been mentioned under the sulphatides, represents an essential constituent of the medullary sheath. The proportion in which it occurs in the sheath can be considered as a measure of the maturity of the sheathing substance.

**THE PROTEIN S AND NEUTRAL S** will be considered together as they bear an important relation to one another and as the combinations in which they occur are essential constituents of all living cells. As has already been stated, the study of these two groups of sulphur compounds gives us a means of investigating the protein metabolism of the central nervous system during its growth period.

TABLE I.

*A comparison of neutral sulphur with protein sulphur in the brain of the albino rat at different ages (figures in per cent of total sulphur).*

	PROTEIN SULPHUR	NEUTRAL SULPHUR
1 day.....	30.5	48.2
10 days.....	44.2	45.4
20 days.....	56.4	28.6
40 days.....	63.7	18.2
120 days.....	61.8	18.7
210 days.....	63.8	14.5

During the early stages when growth is proceeding rapidly and chemical activities may be considered to be at their height, the proportion of



non-colloidal, relatively smaller, neutral sulphur molecules is at a maximum. This is what we should expect when we consider living matter not as a collection of highly organized molecules, but rather as a heterogeneous substratum in which relatively smaller molecules or their dissociated products are engaged in chemical transformations. As the tissues grow and become more highly differentiated and mature, more and more protein is laid down as structural material, and the proportion is shifted in the direction of the protein sulphur. A comparison of the cortex of the human at two years and at maturity illustrates this point.<sup>16</sup>

2 years' cortex.....	Protein S, 63; Neutral S, 22.
19 years' cortex.....	Protein S, 73; Neutral S, 12.

The change suggests, therefore, a decrease in chemically active material associated with the increasing complexity of the tissue. Such data as we have at hand indicate that we have in the protein sulphur and neutral sulphur ratio a valuable means of measuring the relative growth intensity of the nervous system at different periods during its development after the state of cell division has practically ceased.

There might be another way of measuring this intensity of chemical activity, namely, by means of the inorganic sulphates, which represent the end products and the final state of oxidation of the compounds involved in these reactions, but they are eliminated rather easily from the cell, and it is therefore difficult to attach any significance to their variations.

#### *Distribution of phosphorus.*

**Chemistry.** **PROTEIN P.** This group represents phosphorus largely in combination as nucleic acid. In the nervous system this nucleic acid is combined with such a very large amount of protein<sup>17</sup> that the per cent of phosphorus in the resulting nucleoprotein drops to 0.57 per cent as compared with 3 to 4 per cent in such a tissue as the pancreas.

**LIPID P.** Already discussed under phosphatides.

**WATER-SOLUBLE P.** This group includes non-colloidal, water-soluble organic combinations of phosphoric acid and inorganic phosphates. On account of the relative ease with which the organic extractive combinations of this form of phosphoric acid break down, it is difficult to estimate the proportion which is in organic combination. The results which are so far recorded represent, therefore, rather the possible maximum, than a very close approach to the actual value. (See articles of Grindley,<sup>18</sup> Trowbridge,<sup>19</sup> and Forbes.<sup>20</sup>)

<sup>16</sup> Koch, W. and Mann, S. A.: *Journ. of Physiol.*, xxxvi, p. 2, 1907.

<sup>17</sup> Also accounts for poor staining reaction of neurone nucleus.

<sup>18</sup> Grindley: *Journ. of Amer. Chem. Soc.*, xxviii, pp. 25-63, 1906.

<sup>19</sup> Trowbridge, P. F. and Francis, C. K.: *This Journal*, vii, pp. 481-501, 1910.

<sup>20</sup> Forbes, E. B.: *Ohio Agric. Exp. Sta. Bulletin* 215, 1910, pp. 459-489.

*Anatomical distribution and physiological significance.* The protein phosphorus is largely associated with the nucleic acid of the nucleus. In the nervous system it is also supposed to be associated with the Nissl substance, but this is still a doubtful matter. The accuracy with which we can estimate nuclear material in the anatomical sense from such a figure as the protein phosphorus is difficult to determine, as there are three complicating factors.

1. The possibility that the nucleus, as an anatomical unit, contains other compounds besides nucleoproteins.

2. The fact that nucleic acid itself may be associated with very widely varying quantities of protein.

3. The possibility that substances yielding a protein phosphorus fraction may occur in the cytoplasm.

Observations by Miescher<sup>21</sup> on the sperm, however, very strongly suggest that protein phosphorus is largely associated with the nucleus, while lipid phosphorus is largely associated with the cytoplasm.

As regards the *water-soluble phosphorus*, the principal point of interest, just as in the case of the neutral sulphur, is its ratio to the protein or colloidal forms of phosphorus. Thus in a study on a lower plant form (*Aspergillus niger*) Koch and Reed<sup>22</sup> could demonstrate that under extreme conditions, such as can only be realized with plant material, it is possible to carry the growth processes to such a point that all the non-colloidal, water-soluble phosphorus is converted into colloidal combinations. At such a point the growth of the plant comes to a stop.

The function of the *inorganic phosphates* in maintaining the neutrality of protoplasm as suggested by Henderson<sup>23</sup> is also a point of interest, although of less importance to the nervous system than to muscle tissue.

#### *Inorganic constituents.*

*Chemistry.* The inorganic constituents found in the nervous system are the cations Na, K, Ca, Mg, Fe, and the anions Cl, SO<sub>4</sub>, PO<sub>4</sub>.

In the method devised for this study the usual method of estimating these constituents by the ash was abandoned on account of the fact that by the process of ashing, the relation of cations to anions is profoundly altered. As a result of this precaution, the interesting fact has been clearly demonstrated that a large proportion of the cations, especially sodium and potassium, occur combined with complex anions, sometimes colloidal in nature.

The work of Pike<sup>24</sup> has brought to light the interesting point that in the nervous system the sodium and potassium, more especially the latter,

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<sup>21</sup> Miescher, F.: *Hoppe-Seyler's Med.-chem. Unters.*, p. 452.

<sup>22</sup> Koch, W. and Reed, H. S.: *this Journal*, iii, p. 49, 1907.

<sup>23</sup> Henderson, L. J.: *this Journal*, vii, pp. 29-35, 1910.

<sup>24</sup> Koch, W. and Pike, F. H.: *Journ. of Pharm. and Exp. Ther.*, ii, pp. 245-248, 1910.



are combined with such lipoids as the sulphatides and kephalines (a subgroup of phosphatides), while the Ca and Mg have more tendency to remain combined with the proteins.

*Anatomical distribution and physiological significance.* Very little is known of the anatomical distribution of the salts except as shown by the work of Macallum<sup>25</sup> which demonstrates that chlorides and potassium are associated with the nerve fiber. According to Alcock,<sup>26</sup> potassium is supposed to play an important rôle in the propagation of the nerve impulse.

With this introductory statement of the anatomical distribution and physiological significance of the substances quantitatively determined, we may now present the results of a study of their variation during the growth of the brain.

The brain of the albino rat was selected for this study, for the reasons already presented in the first paper of this series.<sup>27</sup>

From a comparison of the brain of the albino rat at birth and the brain of the fetal pig, it was found that the brain of the new born rat is as young nervous material as can conveniently be analyzed at present. It forms therefore a suitable starting point for this study of chemical differentiation during growth. The analyses reported in this paper are those of the brains of rats aged respectively, 1, 10, 20, 40, 120, and 210 days. The results show that it was possible to follow closely the various structural changes which occur during the differentiation of the growing nervous system

The material was furnished by the Wistar Institute of Anatomy; the brains being collected and analyzed in the manner already detailed.<sup>28</sup> Koch's quantitative methods were used.<sup>29</sup>

#### RESULTS OF ANALYSES.

The results of analyses are embodied in Table II. Duplicate analyses have been carried on throughout, and are summarized in Table III. This table gives the averages of the analyses, except

<sup>25</sup> Macallum, A. B.: *Journ. of Physiol.*, xxxii, pp. 95-128, 1905; Macallum, A. B. and Menten, M. L.: *Report 75th Meeting British Assoc. Adv. Sci.*, p. 555, 1906.

<sup>26</sup> Alcock, N. H.: *Journ. of Physiol.*, xxxix, pp. 402-410, 1911.

<sup>27</sup> Koch, Mathilde L.: *this Journal*, xiv, pp. 267-279, 1913.

<sup>28</sup> Koch, Mathilde L.: *loc. cit.*

<sup>29</sup> Koch, W.: *Journ. of Amer. Chem. Soc.*, xxxi, pp. 1335-1364, 1909.



in two instances where the value from one analysis only is preferred: Table IV gives the absolute weights of these constituents, as found in one brain; while Table V gives the ratio of increase of the different constituents, taking the amount of each constituent in the brain of the rat at birth to be unity and determining the number of times each constituent had increased at successive ages from birth to maturity. For comparison there is given in Table II one analysis of the spinal cord at 120 days.

#### DISCUSSION OF RESULTS.

The growth of the nervous system from the first laying down of the neural canal to maturity may be divided into four periods. The first period, during which cell division is the most characteristic feature, lasts to about birth. A short time before birth cell division begins to decrease. The chemical changes during this first period were not studied directly in the albino rat for the reasons stated in the first paper<sup>30</sup> but the composition of the nervous system in this primitive, undifferentiated state may be seen in the analysis of the fetal pig brain reported in the first paper of the series. At this time phosphatides are present, sulphatides are relatively less important and cerebrosides are entirely lacking; proteins, phosphatides, extractives, salts and water are the predominant constituents of the tissue.

The second period (see Table VI) lasts from birth for about ten days, when the third period begins. The second period is characterized structurally by the development of fibers from the cells and the increase in their size. Donaldson has estimated that the number of nerve cells does not increase more than 3 to 6 per cent during this period, but the cells do add to the number and size of their branching processes. This period, as may be seen from Table VI, is one of intense growth of all the solid constituents. The proteins continue throughout this period to be formed at a very rapid rate, 4-5 mgms. being laid down per day. Cerebrosides are either absent entirely, or present in very small quantities.

In the third period, that of most rapid growth, from the tenth to the twentieth day, medullation begins. There is a wonderful

<sup>30</sup> Koch, M. L.: this *Journal*, xiv, p. 279, 1913.

TABLE II.

*Chemical composition of the brain of the albino rat at different ages.*  
*Cord of the albino rat.*  
*(Inserted for comparison.)*

Age in days.....	1	10	20	40	120	210	120
Body weight in grams.....	5.5	12	20	43	182	112.3	112.3
Moist weight of one brain in grams	0.25	0.860	1.228	1.329	1.659	1.551	0.365
Solids in per cent.....	10.42	14.7	17.5	20.1	21.6	21.9	27.1
Dry weight of one brain in grams.	0.026	0.127	0.215	0.233	0.358	0.336	0.099
Number o' brains in sample.....	100	40	48	59	30	31	90
Laboratory number.....	W. 16	W. 39	W. 17	W. 25	W. 7	W. 8	W. 9

*Constituents in per cent of solids.*

Proteins.....	58.2	58.3	56.4	56.5	53.9	52.7	48.7	48.1	47.2	48.0	48.5	32.8
Phosphatides.....	14.8	15.6	10.6 (?)	12.3	21.1	21.7	20.5	23.2	21.9	21.3	22.0	25.3
Ce ebrosides.....	*	*	*	*	3.1	2.9	6.3	5.5	6.6(?)	8.4	8.4†	12.5
Su'phatides.....	1.5	1.4	0.73(?)	2.6	2.4	2.6	2.7	2.4	3.5	3.6	4.5	7.0
Organic extractives.....	16.5	19.3	19.3	15.1	13.8	15.3	13.8	15.9	9.7	9.8	9.8†	7.6
Inorganic constituents.....												
Cholesterol (undetermined) †.....	9.0	5.4	13.0	13.5	5.7	4.8	8.0	4.9	11.1	8.9	6.8	14.8
Total su'phur.....	0.96	1.04	0.72	0.83	0.69	0.70	0.58	0.52	0.55	0.57	0.58	0.45
Total phosphorus.....	1.82	1.92	1.28	1.48	1.66	1.67	1.55	1.50	1.40	1.44	1.39	1.44



TABLE II. Continued.

*Distribution of sulphur in per cent of total S.*

Protein S.....	31.1	30.0	48.6	44.2	57.5	55.3	65.1	62.4	61.2	62.4	63.8	53.7
Lipoid S.....	3.2	2.8	2.2	6.1	6.7	7.5	9.2	10.1	12.8	12.5	15.6	30.9
Neutral S.....	49.1	47.3	45.1	45.4	29.7	27.5	17.0	19.3	19.2	18.3	14.5	10.3
Inorganic S.....	16.6	19.9	4.1	4.3	6.1	9.7	8.7	8.2	6.8	6.8	6.1	5.1

*Distribution of phosphorus in per cent of total P.*

Protein P.....	13.3		13.0	13.9	6.0	5.8	9.9	7.5	7.4	7.3	6.8	5.6
Lipoid P.....	33.2	33.0	33.8	36.1	52.2	53.5	56.1	58.5	65.8	62.3	67.6	77.4
Water Sol. P.....	53.5	53.6	53.2	50.0	41.8	40.7	34.0	34.0	26.8	30.4	25.6	17.0

\* Cerebrosides not determined in brains at birth and 10 days. Probably none present at this age.

† By difference.

‡ Indicates doubtful result.

† Taken from W. 8.

TABLE III.

*The relative proportions of the constituents of the brain of the albino rat at different ages (averages from Table II).*

	AGE IN DAYS					
	1	10	20	40	120	210
Moist weight of one brain in grams..	0.25*	0.86†	1.28*	1.38*	1.60*	1.67†
Solids in per cent.....	10.42	12.5	17.5	20.34	21.65	21.9
Dry weight of one brain in grams....	0.026	0.107	0.224	0.281	0.347	0.365
Number of brains in each sample....	100	40	54	35	30	31
Laboratory Number.....	W. 16, 24	W. 40	W. 17, 25	W. 28, 29	W. 7, 8	W. 13

*Constituents in per cent of total solids.*

Proteins.....	58.25*	56.5†	53.3*	48.4*	47.6*	48.5†
Phosphatides.....	15.2	12.3	21.4	21.8	21.6	22.0
Cerebrosides.....			3.0	5.9	8.4†	8.4†
Sulphatides.....	1.45	2.6	2.5	2.55	3.55	4.5
Organic extractives.....	17.9	15.1	14.55	14.85	9.75	9.8†
Inorganic constituents.....						
Cholesterol (undetermined)§.....	7.2	13.5	5.25	6.5	9.1	6.8
Total sulphur.....	1.00	0.83	0.70	0.55	0.56	0.58
Total phosphorus.....	1.87	1.48	1.66	1.52	1.42	1.39

*Distribution of sulphur in per cent of total S.*

Protein S.....	30.5	44.2	56.4	63.75	61.8	63.8
Lipoid S.....	3.0	6.1	7.1	9.65	12.7	15.6
Neutral S.....	48.2	45.4	28.6	18.15	18.7	14.5
Inorganic S.....	18.3	4.3	7.9	8.45	6.8	6.1

*Distribution of phosphorus in per cent of total P.*

Protein P.....	13.3	13.45*	5.9	8.7	7.3	6.8
Lipoid P.....	33.2	34.95	52.85	57.3	64.1	67.6
Water sol. P.....	53.5	51.6	41.25	34.0	28.6	25.6

\* Record from average duplicate analyses.

† Record from one analysis only.

‡ Taken from analysis, W. 8.

§ Obtained by difference.



TABLE IV.

*Absolute weights, in milligrams, of the constituents of a single brain of the albino rat at different ages (prepared from Table III).*

	AGE IN DAYS					
	1	10	20	40	1 20	210
Moist weight of one brain in grams....	0.25	0.86	1.28	1.38	1.60	1.67
Solids in per cent....	10.42	12.5	17.5	20.34	21.65	21.9
Dry weight of one brain in grams....	0.026	0.107	0.224	0.281	0.347	0.365
Laboratory Number....	W. 16, 24	W. 40	W. 17, 25	W. 28, 29	W. 7, 8	W. 13

*Absolute weights in milligrams.*

Proteins (1)†.....	15.14*	60.45†	119.4*	136.0*	165.2*	177.0†
Phosphatides (2)...	3.95	13.16	47.9	61.3	74.95	80.3
Cerebrosides (3)....			6.7	16.6	29.15	30.66
Sulphatides (4).....	0.38	2.78	5.6	7.2	12.3	16.4
Organic extrac- tives.....	4.65	16.16	32.6	41.7	33.8	35.8
Inorganic constit- uents.....						
Cholesterol unde- termined (5)....	1.87	(14.45)	11.7	18.2	31.6	24.8
Total sulphur.....	0.26	0.90	1.57	1.54	1.94	2.12
Total phosphorus...	0.48	1.6	3.72	4.30	4.93	5.07

*In absolute weight in milligrams of sulphur.*

Protein S (1S)§.....	0.079	0.398	0.885	0.982	1.199	1.352
Lipoid S (4).....	0.008	0.054	0.111	0.149	0.246	0.330
Neutral S (6).....	0.125	0.409	0.449	0.279	0.363	0.307
Inorganic S (7).....	0.047	0.039	0.122	0.130	0.132	0.129

*In absolute weight in milligrams of phosphorus.*

Protein P (1P).....	0.064	0.215*	0.220	0.374	0.360	0.345
Lipoid P (2).....	0.161	0.558	1.964	2.464	3.160	3.427
Water sol. P (8)....	0.260	0.826	1.532	1.462	1.410	1.298

\* Record from average duplicate analyses.

† Record from one analysis.

‡ Figures in parentheses in this section refer to Chart III.

§ Figures in parentheses in this and the following sections refer to Chart IV.

TABLE V.

*The ratio of the increase of the constituents of the brain of the albino rat at different ages, taking the amount of each constituent found in the brain at birth as unity (prepared from Table IV).*

	AGE IN DAYS					
	1	10	20	40	120	210
Total Solids.....	1	4.0	8.6	10.8	13.3	14.0
Proteins.....	1	4.0	7.9	9.0	11.0	11.7
Phosphatides.....	1	3.3	12.0	15.5	19.0	20.3
Cerebrosides.....						
Sulphatides.....	1	7.4	14.8	19.0	32.6	43.5
Organic extractives.....	}	3.5	7.0	8.9	7.2	7.7
Inorganic constituents.....						
Cholesterol (undetermined).....	1	7.7	6.2	9.0	16.9	13.2
Total sulphur.....	1	3.4	6.0	5.9	7.4	8.0
Total phosphorus.....	1	3.2	7.6	8.8	10.1	10.4
Protein S.....	1	5.0	11.1	12.3	15.0	17.0
Lipoid S.....	1	6.9	14.3	19.1	31.5	42.3
Neutral S.....	1	3.3	3.6	2.2	2.9	2.5
Inorganic S.....	1	(1.3)	2.6	2.7	2.8	2.7
Protein P.....	1	4.1	4.3	7.2	7.0	6.7
Lipoid P.....	1	14.3	15.3	19.2	24.7	26.8
Water sol. P.....	1	3.9	7.4	6.9	6.8	6.2

TABLE VI.

*Rate of growth (milligrams formed per day) of different constituents in a single brain of the albino rat at different age periods (prepared from Table IV).*

	AGE PERIODS				
	2	3	4		
Between.....	1-10 days	10-20 days	20-40 days	40-120 days	120-210 days
Proteins.....	4.53	5.9	0.84	0.36	0.13
Phosphatides.....	0.92	3.5	0.67	0.17	0.06
Cerebrosides.....			0.49	0.15	0.006
Sulphatides.....	0.24	0.29	0.08	0.06	0.045
Organic extractives.....	}	1.51	0.46	0.00	0.000
Inorganic constituents.....					
Cholesterol (undetermined).....	(0.49)	(0.49)	0.32	0.17	0.000

outburst of activity in forming phosphatides which reach a maximum rate of formation of 3.5 mgms. per day. This change is no doubt correlated with the great growth of the fibers and the beginning of medullation. The organic extractives and inorganic constituents continue to be formed at the same rate since the cell bodies are increasing in size; probably not more than from 10 to 20 per cent having reached anything approaching adult size up to this time.

The sulphatides, although present in less quantity than the phosphatides, reach also their maximum rate of formation. The whole chemical picture is that of a rapid growth of protoplasm, with a change in its character owing to the increase of phosphatides. During this period the neurones increase rapidly in size.

Attention is particularly directed to the temporary great increase in neutral sulphur during these two periods of intense growth (Table IV). The significance of this has already been discussed on p. 431 *et seq.*

The fourth growth period is the period of continued medullation. This period is characterized chemically by a great reduction in the rate of formation of all substances except the cerebro-sides. These latter between 20-40 days come into view, almost equalling the phosphatides and being more than half the amount of the proteins formed at the same time. The cerebro-sides contribute a large share toward medullation. The rate of formation of the various constituents per day falls in the 20-40 day period, as compared with the 10-20 day period, in the case of the proteins to one-seventh; the phosphatides to one-fifth; the sulphatides to one-third; and the organic and inorganic extractives to one-third. The formation of the proteins decreases the most; the cerebro-sides, the least. If the rate of formation in the 40-120 day period is compared to that of the 10-20 day period it is seen that the protein formation has decreased to one-sixteenth; the phosphatides to one-twentieth; the sulphatides to one-fourth but are still increasing. On the other hand, the organic extractives and inorganic constituents have not increased at all, indicating that metabolism is much reduced in its rate and the growth of the protoplasm is much slower. During this fourth period then, the sulphatides continue to be formed at a more rapid rate, relative to their total amount, than any other constituents; and in the 120-210



day period, the total sulphatides formed surpass the cerebroside, nearly equal the phosphatides, and are more than one-third the proteins. The constant production of sulphatides is, therefore, a marked feature of late medullation, just as that of the phosphatides is of the early medullation. The sulphatides diminish in their rate of formation far less than any other constituents.

Finally we have the period from 210 days on: the period of stationary or adult life. We have no definite chemical data as to any changes occurring during this period, but from such data at hand, as the periods just studied, we can assume that the growth processes during adult life are practically stationary except perhaps a very gradual increase in the per cent of solids.

The enlargement of the brain may, therefore, in great part be accounted for chemically by the formation of the medullary sheath. Donaldson<sup>31</sup> has found that some 88 per cent of the volume of the adult brain is composed of the axons and their sheaths, while the cell bodies with their dendrites and the supporting tissues together only make up the remaining 12 per cent. The axones, therefore, medullated or non-medullated, are mainly responsible for the increase of the size of the brain and for the changes which it undergoes during post natal growth. To bring more vividly before the eye the relative rate of growth of the various constituents, we have prepared Charts 1 and 2 from Tables III and IV. These charts are self explanatory. We have also prepared Charts 3 and 4, an explanation of which is given below.

Chart 3 shows the relation of lipid<sup>32</sup> to protein. In this the weights of the several constituents are represented for one brain at each age. This chart shows that while both the proteins and the lipoids are increasing in absolute weight, the proportion of lipid to protein is becoming greater and greater as the tissue grows older. This indicates that the rate of increase for the lipoids is greater than for the proteins (also brought out in Table VI). At 120 and at 210 days we find that the lipoids and pro-

<sup>31</sup> Donaldson, H. H.: *Journ. of Nervous and Mental Disease*, xxxviii, p. 260, 1911.

<sup>32</sup> Lipoids here include the phosphatides, cerebroside, and sulphatides; cholesterol, which is classed as lipid, is here recorded in the "undetermined."

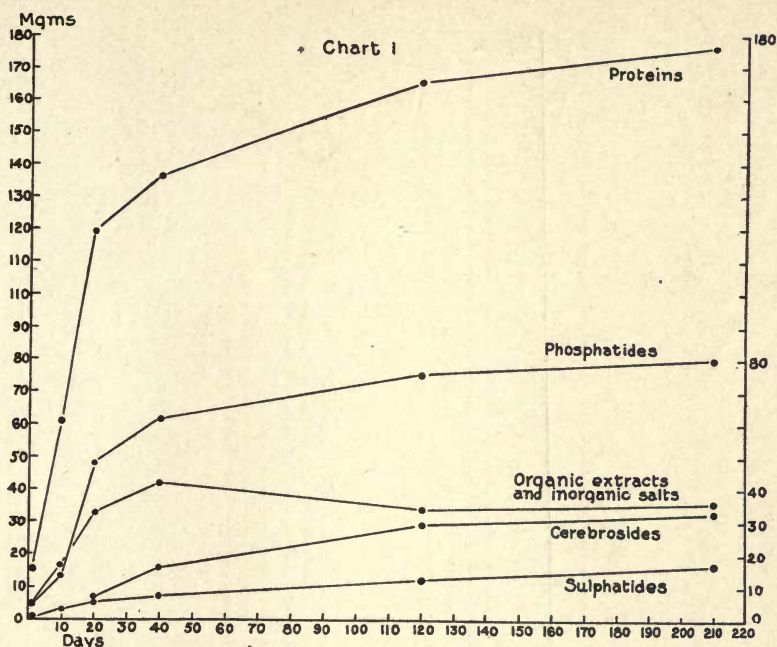


CHART 1. Shows the absolute weight in milligrams, of the constituents of a single brain of the albino rat at different ages. (Age in days along the abscissa; mgms. on the ordinate.)

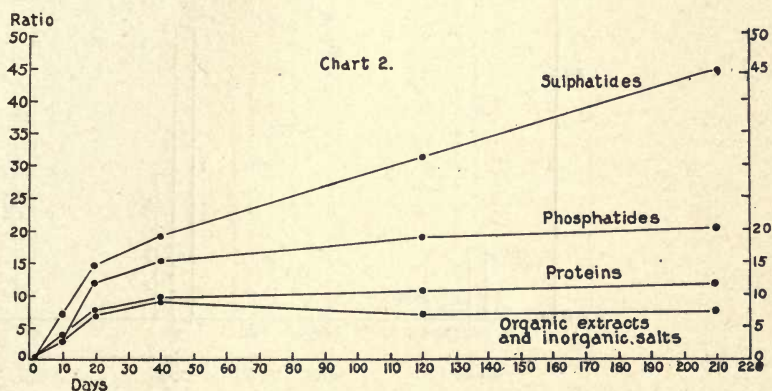


CHART 2. Shows the rate of increase of the different constituents of the brain of the albino rat at different ages, taking the amount of each constituent in the brain at birth as unity. (The ordinate shows how many times the weight of each constituent has increased over its amount at birth at the age plotted on the abscissa.)

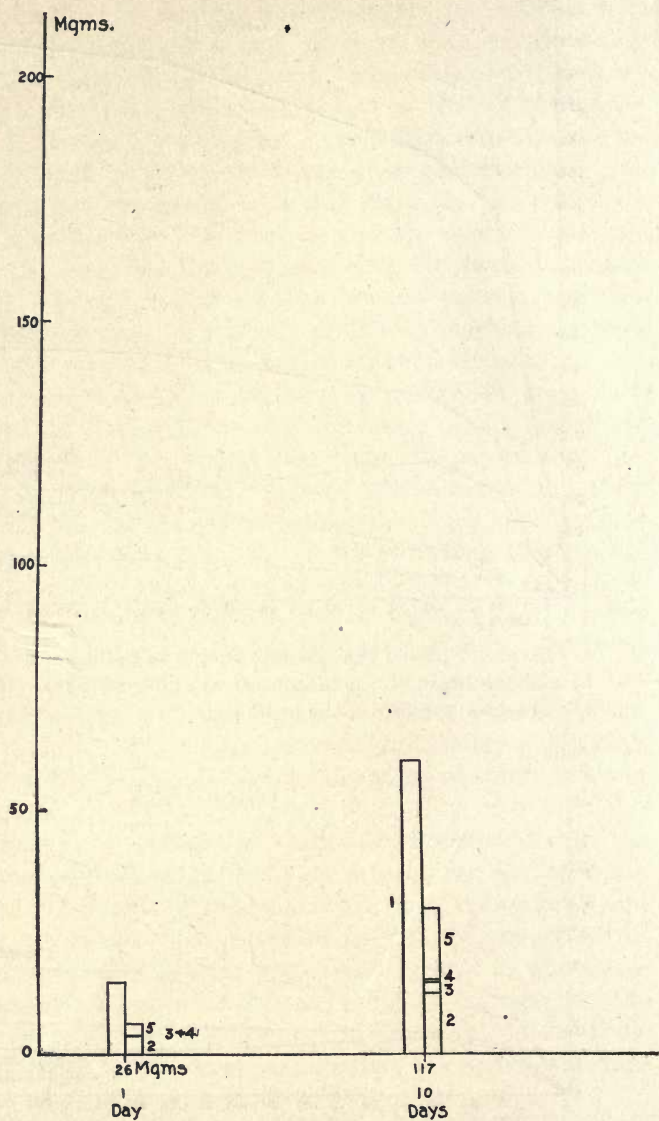
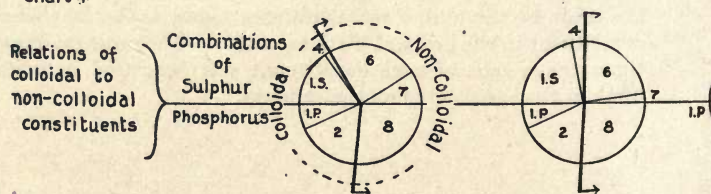


Chart 4





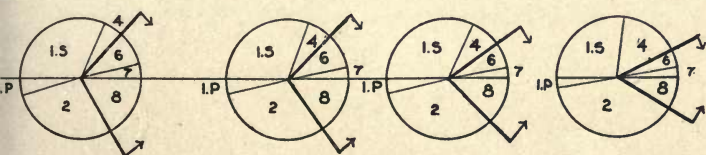
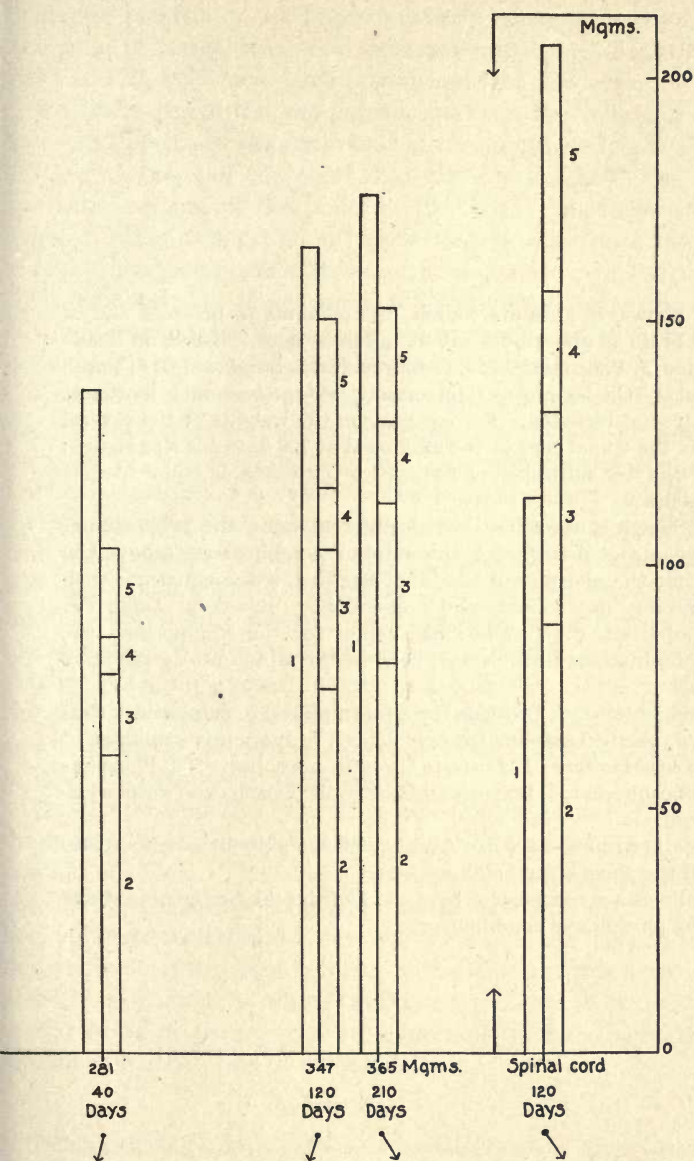


CHART 3. Shows, in absolute weight, the amounts of proteins and of lipoids in the brain of the albino rat at different ages. Based on Table IV. 1, Proteins; 2, Phosphatides; 3, Cerebrosides; 4, Sulphatides; 5, Undetermined lipoids (Cholesterol). The organic extractives and inorganic constituents are not included. For comparison the weights of the several constituents in the spinal cord of the albino rat at 120 days are also shown; the weight of the dry substance of the cord being taken as equal to that of the 120-day brain.

CHART 4. Shows, in the albino rat at different ages, the proportional values in segments of a circle for the sulphur combinations (above the equator) and for the phosphorus combinations (below the equator); with a further grouping into colloids and non-colloids. Based on Table IV. Again for comparison the proportional values for the sulphur and the phosphorus combinations in the spinal cord of the albino rat at 120 days are also given.

*Sulphur combinations:* 1, Proteins (protein sulphur); 4, Sulphatides (lipoid sulphur); 6, Neutral sulphur (proteic acids); 7, Inorganic sulphates.

*Phosphorus combinations:* 1, Proteins (protein phosphorus); 2, Phosphatides (lipoid phosphorus); 8, Organic and inorganic phosphates (water soluble phosphorus).

The colloids are represented by 1 and 4, of the sulphur combinations and 1 and 2 of the phosphorus combinations.

The non-colloids are represented by 6 and 7 of the sulphur combinations and by 8 of the phosphorus combinations.

teins are present in the brain in nearly equal proportions. For the sake of comparison, the corresponding values for the spinal cord at 120 days have been introduced into this chart.

To make easier the comparison between the relations of proteins and lipoids in the brain and in the spinal cord, it is assumed for the purposes of the chart that the dry weight of the cord is the same as that of the brain at 120 days. Since the cord contains a larger proportion of white matter than does the brain, we find that the lipoids in this case predominate over the proteins. This indicates that the chemical differentiation during the growth of the nervous system, as recorded in this paper, is largely concerned with the development of the medullated nerve fiber.

Chart 4 shows very strikingly the great decrease with advancing age in the non-colloidal contrasted with the corresponding increase in the colloidal sulphur and phosphorus compounds. Particular attention is called to the neutral sulphur which in the young, rapidly metabolizing tissue constitutes the greater proportion of the total sulphur, whereas it becomes extremely small at 210 days when growth metabolism is at an end. Evidently this fraction may, with reserve, be considered an index of growth metabolism. With advancing age the colloidal, less active, substances gradually crowd out the non-colloidal. This is in striking accord with the interesting suggestions of Child<sup>33</sup> that senescence is due to the accumulation of these colloidal solids, which interpose resistance to metabolism.

Finally we find the growth process characterized by a steady diminution in the proportions of water and an increase in the proportion of solids. This change is due not alone to medullation in the strict sense, since as Donaldson<sup>34</sup> has pointed out the decrease begins before medullation, between birth and ten days in the rat. He attributes it to a rapid growth of the axone at this time. Water and the proportion of neutral sulphur are therefore criteria of the youthfulness of tissue, while the increase of lipoid sulphur (sulphatides) is a criterion of medullation.

<sup>33</sup> Child, C. M.: *Archiv. f. Entwicklungs-mechanik d. Organismen*, xxxi, p. 571, 1911.

<sup>34</sup> Donaldson, H. H.: *Journ. of Neurology and Psychology*, xx, p. 138, 1910.



## SUMMARY.

The principal results of this study may be summarized as follows:

Well-marked and characteristic chemical changes occur in the rat-brain during its growth and these changes are obviously correlated with its anatomical differentiation.

The principal chemical changes noted are:

1. A general decrease in the per cent of water which is not due entirely to medullation since the decrease begins before medullation (Donaldson '10).

2. A diminution in the relative per cent of protein in the total solids due to the formation of a large amount of lipoid matter.

3. The lipoids which appear coincident with medullation and of which the development is *pari passu* with medullation are the cerebrosides and sulphatides. These, therefore, are chiefly found in the medullary sheaths.

4. There is a great outburst of phosphatide formation at the very beginning of medullation, but the phosphatides are present also in large amounts before medullation. The phosphatides are present, therefore, in the cells as well as the sheaths.

5. The extractives are present in largest amounts during fetal and early life when growth and metabolism are at a maximum. Particularly the water-soluble, organic sulphur compounds (neutral sulphur) diminish relatively with age, while the colloidal sulphur increases. The relations of the neutral sulphur may be interpreted, therefore, as indicating the intensity of metabolic activity.

6. The great increase of colloidal matter with age clearly indicates that this, in the form of supporting structures, constitutes a relatively inactive material which presumably serves to localize chemical processes. The accumulation of this material is probably one factor producing the general slowing of metabolism characteristic of senescence. This would thus become one cause of senescence as Child has suggested.

## ADAPTATION FROM THE POINT OF VIEW OF THE PHYSIOLOGIST<sup>1</sup>

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I FEEL much ashamed in having to expose my intellectual nakedness before the members of this society. When I came to this meeting I supposed that adaptation, or the fitness of organisms to their environments, was a physiological truism; that fishes were fitted by their structures and functions to a life in the water; that frogs were so constituted that they could live either on land or in water; and I was even so ignorant as to believe that many structures of a bird's body adapted it to flight. But it appears from the paper of one of my colleagues that in all of these things I was most woefully mistaken.

I feel some hesitation, also, in appearing before a society composed largely of American students of genetics, for I have no new and confusing terminology to propose; and owing to my ignorance of the language they speak and of the short-hand symbols sometimes employed, I am, perforce, compelled to speak in ordinary English which may be understood by any one; all of which, I fear, must invest all I have to say with an air of superficiality, or even of simplicity. I am besides a confirmed conservative in the matter of evolution, holding fast to the explanation of adaptation given by Darwin of natural selection of small variations; having little or no confidence that genes, unit characters, mutations, saltations, allelomorphs, determiners, inhibitors, dominants and recessives, genotypes and phenotypes, are anything more than ghosts, without substance; and looking always for simple explanations of a physical and chemical kind, capable of

<sup>1</sup> Read at the Symposium on Adaptation at the meeting of the American Society of Naturalists, Cleveland, January 2, 1913.



expression in ordinary language, of the apparent complexities of evolution. I avow myself as a physiologist to be a follower of Darwin, admiring his methods of careful experiment and observation, his long cogitations, and with confidence in the soundness of his judgment. There has been a tendency of recent years in certain quarters to belittle his work, to make fun of his conclusions, to deny that evolution has been a slow and steady continuous process, as the rocks show, and to assert that it has taken place by a hop, skip and a jump, and that it would have taken place anyway without natural selection. Physics and chemistry have attempted to express the physical world in terms of matter and energy, and many biologists are attempting to extend this method to the living world. While this is a necessary and admirable thing to do, it must not be forgotten that in doing so they are neglecting the main fact of life, consciousness, and that the phenomena of life can not be accounted for if this is neglected. It is obvious, too, that the physicist, with his present conception of matter and energy, is making as great a mistake in neglecting the psychical side of matter as the biologist would make if he neglected the physical side. For the psychical, like the physical, must be due to the properties of the atoms, or at least is associated always with them. For the atoms are the same in living and lifeless, their properties are inherent in them and can not be taken away and added to them as if they were wagons, which changed horses, as Du Bois Raymond has put it.

It is my opinion that physiology comes powerfully to the support of Darwin's conclusions; that it shows clearly that there are no such things as independently variable, unit characters; that a jump is a physiological impossibility; and that most so-called mutations are in reality reversions, as Darwin thought; and in this position physiology is, I believe, supported by paleontology.

But while accepting many of Darwin's conclusions, we must all admit that many phenomena are very hard



to understand on the basis of Darwin's explanations. Among these difficulties, most of which were recognized by Darwin, there are the phenomena of parallel evolution among different species and genera, which, though diverse, appear all to be moving forward in the same direction; the phenomena of steady, limited progress in one direction which point toward orthogenetic variation; the phenomena of the appearance of rudiments and their development until useful. It is exactly these difficulties upon which physiology throws some light; and it is of them that I particularly wish to speak.

In the evolution of animals two movements may be perceived: a spreading out and a progress; a diversification and a movement forward. The first movement is illustrated by the formation of many different species in one genus; or of many genera of the same type of animal; the second by the movement forward in the line of evolution of all these species. These two movements were not sharply distinguished by Darwin, but they have been more or less clearly recognized by several philosophers. It is this double movement which has given the animal kingdom the form of a branching tree instead of a single trunk. Darwin dealt mainly with the first of these movements, which gives rise to genera, species and varieties; which is shown by the diversification of animals and plants in domestication by human selection; and he explained it by the progressively better adaptation of forms to particular environments. He believed the second movement, the movement upward, was due to the same cause.

It is the second movement which has been so hard to explain and which has particularly puzzled the paleontologist; the successive series of dominating types on the earth's surface culminating in man; the progress steadily toward the goal of consciousness and intelligence.

The question which I wish to raise is whether these two movements, which are at right angles to each other, may not be due to the natural selection of two different kinds

of adaptations: first, adaptations of form and function to different kinds of environments; and second, the natural selection of the function of irritability, or, in other words, to the selection of adaptability, or the adaptation to changeableness of environment. Selection of the first kind of adaptations may have given rise to varieties, species, genera of the same type of animal, and have produced the spreading, or diversification; while selection of the second kind of adaptation may have produced the movement onward and upward of all animal forms.

These two kinds of adaptations do not always go together and selection of the one may outweigh the other. It is because selection to a specific environment sometimes is more important than selection of adaptations to changeableness, that not all organisms have progressed in the scale of evolution equally rapidly: but some have persisted in special environments with slight changes of structure for very long periods, or may even have retrogressed; while other forms, in which the second adaptation has been rigorously selected, have moved rapidly onward and upward, and show little adaptation to any special environment.

The question whether evolutionary progress is due to the selection of this second adaptation, that of adaptability, occurs very naturally to a physiologist, because, in the first place, the evolutionary development of consciousness and intelligence appears to him to be one of the most important, if not the most characteristic movement in evolution; and in the second place, his point of view in considering evolution and adaptation is somewhat different from that of the zoologist or the paleontologist. To him the organism does not appear constituted of bones, skin, horns, or other structures, but to be constituted essentially of a number of mechanisms in activity, each mechanism having a definite function to perform. Evolution, for the physiologist, is not evolution of structure primarily, but evolution of function; and he natu-



rally expects to find that the adaptations of function have been of great importance in determining survival.

Of all the physiological properties of the original protoplasm upon which natural selection might be supposed to act, irritability, the most fundamental property of living matter, would seem the most probable point of attack; for irritability is that property of protoplasm in virtue of which it adjusts itself to its environment. It is the property of response; and since it is the environment which is acting as the judge of the excellence of the response and doing the selecting, it would seem that it must be upon this property that all organisms must be tested. It is, moreover, this property that Spencer has very acutely selected as the most fundamental characteristic of living organisms, namely, the power of continuous adjustment of internal to external conditions. It would seem probable that however well animals might be adapted to special environments by the action of natural selection, this particular property, or function, which has to do with the continuous adjustment of internal to external relations must have been throughout the whole course of evolution of predominant importance. And if there has been any unity in the progress; if the course of evolution has been at all in any single direction; and if the natural selection theory is true; it must be in the direction of the perfecting of this function.

I think this short statement will make it clear why the physiologist turns naturally to this fundamental quality, or property of living things, when he considers evolution and adaptation; for however organisms may vary in structure or other particulars, they all have irritability in common. Moreover, I think most physiologists will agree with me that this particular property has been too often neglected by most students of evolution, among whom physiologists have been unfortunately very rare.

Irritability shows itself in all cells by the power of internal change in response to an external change. In most cells of the body there is nothing especially adaptive



in the nature of many of these responses; but it is quite otherwise, if we consider the organisms as wholes. It is clear that all organisms have not only the power of reacting to an external change, but many of their reactions are adaptive to a surprising degree. This is indeed the very crux of the difference between living organisms and lifeless things. A lifeless thing can not adjust its internal to its external relations so that it can continue to exist in a changed environment. A crystal in a solution of its kind must dissolve, if the concentration is kept ever so little below saturation; a whole universe must pass away, if anywhere within it there is a persistent uncompensated difference of potential. With living things it is quite otherwise. They have the power of interposing resistances to the potential difference. All living things without exception have adaptive responses so that they are able to continue in existence even though their surroundings change in many different ways. They possess adaptability. Their responses due to their irritability are adaptive responses. The irritability of the organism as a whole is, then, above everything else characterized by power of adaptive response.

It is not difficult to imagine how this specialization of the general property of irritability arose. Some of the indefinite responses of the original organisms to environmental change protected the organism against the change. Organisms with such responses survived and their descendants had the property of a limited adaptive response to this particular change. From this crude beginning further progress was easy. The changes in the environment, though many, are not indefinite in number, and adaptations in the nature of direct responses easily arose and were perfected.

Adaptability, then, appears to the physiologist as the master word of evolution. And many facts also may be urged as confirming this conclusion. For example, one and all of the great physiological mechanisms of the body have a single purpose: to secure adaptability. Not to

adapt an organism to one environment, but to all environments, and thus to make it superior to all environments. Furthermore, the higher organisms are specially remarkable for the development of that master tissue of the body which is preeminently irritable and of which the main function is the adjustment of internal to external relations, the nervous system; and finally that the inference is sound may be concluded from the fact that it is by adaptability and by no other quality whatever that organisms may be arranged in the order of their evolutionary progress.

It is not at all surprising that adaptability should be the most important adaptation in nature, overpowering, except in special cases, and dominating all others. For there is but one certain thing in nature: namely uncertainty. The most constant feature of all environments, but particularly of land environments, has been their inconstancy. Changeableness is the chief characteristic of all environments, whatever their special characters may be. There are changes of light, temperature, climate, oxygen and carbon dioxide, moisture; changes due to the introduction of new species by migration upsetting nature's balance; changes in the food supply. Climates, flora and fauna change; change alone persists. Change is the essential thing. We may expect, therefore, if Darwin be correct in his conclusion that variation and natural selection account for evolution, that adaptation to changeableness must be the chief adaptation in nature, and more than all others, it must have determined the general course of evolution. This is found to be the case and the great physiological mechanisms of the body are designed, as already stated, to subserve this fundamental adaptation. Adaptability is that power which fits organisms to withstand the unexpected: the vicissitudes of life; special adaptations of form and color may contribute to the survival of animals; but the essential, or root, adaptation is to changeableness. By adaptation to all environments they become finally superior to all environments.



Superiority to environment, and not adaptation to it, is secured through the irritability of the organism considered as a whole.

The great mechanisms of the body which have this function are several. First, the heat-regulating mechanism, for by means of this organisms are rendered independent of the temperature of their environments. They can exist in the tropics or in the arctics and withstand the extremes of our own climate, while maintaining their activities. This is a complex mechanism consisting of insulating material in the skin; trophic nerves to the internal organs; a closed vascular system; a power of rapid oxidation; supra-renal capsules; pancreas; nervous coordination; sweat glands; evaporation of water in the lungs; temperature nerves. More than any other this mechanism enabled the mammals to conquer the reptiles and supplant them. The mammals became independent of the temperature of their environments. A mechanism not coming by jumps, but the rudiments found far down in the fishes and slowly evolved.

A second fundamental mechanism of great importance for the mammals in supplanting the reptiles and other animals probably was that concerned in immunity. Most of the toxins of poisonous reptiles are of a protein nature. The mammals have developed a mechanism, the details of which are still obscure, but which apparently consists in the conversion of these protein toxins into bodies which neutralize the toxins from which they are formed, that is, into antitoxins. We find, as a matter of fact, that at least many of the mammals are able apparently to make an anti-toxin out of any kind of a foreign protein. Besides this mechanism of defense, useful against bacteria, as well as against snakes, there is the primitive mode of phagocytosis and the chemical method of defense, which consists either in the prevention of absorption, or in the chemical neutralization of the poison by union with other substances. Thus the toxicity of phenols, benzoic acid and many alkaloids are neutralized. By this mechanism



mammals are rendered superior to the attacks of many of their enemies and to this extent rendered superior to their environments.

Third, there is the mechanism for rendering mammals tolerably independent of the moisture content of their environment, a mechanism most highly developed in the reptiles. A mechanism formed by the replacing of the wet skin of the amphibian by a dry or scaly skin; the perfecting of the kidneys to maintain osmotic pressure of the blood; the control of the sweat glands and loss of water by the intestines; the development of membranes non-permeable to salts, so that animals may sit in fresh water and not lose their salts. One of the most interesting parts of this mechanism is shown in the reptiles and birds, in the substitution of uric acid for urea in their excretions. By this improvement reptiles have secured almost complete independence of the water content of their environments. They make enough water in their own bodies to supply their small losses. This again is a mechanism of which we can trace the steady growth without a break from the invertebrates to man.

A fourth great mechanism makes mammals independent of barometric fluctuations and less dependent on a fixed atmosphere. By means of their blood loaded with hemoglobin carried in corpuscles lacking all oxygen-consuming power, they are able to live on lofty plateaus, or in deep valleys; and in the presence of much or little carbon dioxide.

The mechanisms having to do with reproduction and the caring for the young afterward have this same advantage of rendering the mammals independent of environment.

A sixth mechanism is the alimentary mechanism, most highly perfected in man. This has rendered him independent of any particular kind of food. He can make his body of any kind of plant or animal. He can make carbohydrate out of protein and many other things. He can live in any climate largely because of this mechanism.

Again a complex mechanism, consisting of teeth, of digestive glands tearing proteins and carbohydrates to pieces, so that he can build up his own proteins from any other kind, useless amino acids being converted into sugar and urea.

The last and by far the most important of these great mechanisms of adaptability is that which provides for every contingency; for the unexpected. It seems that nature, after elaborating these other mechanisms to meet particular vicissitudes, has lumped all other vicissitudes into one and made a means of meeting them all. One can not but be pleased by the apparent ingenuity of this solution. I refer to the nervous mechanism. It is obvious how this mechanism, by substituting choice for blind instinct, consciousness for unconsciousness, developing memory, so that one can profit by experience, and intelligent adaptation of means to ends, has provided finally for all possible contingencies of the future. She has spoken her last word. Adaptability, or superiority to environment, was the end so blindly sought; memory, consciousness, choice were the means, shall I say the means as blindly adopted?

To the physiologist, then, adaptability appears to be the touchstone with which nature has tested each kind of organism evolved; it has been the yard stick, with which she has measured each animal type; it has been the counterweight against which she has balanced each of her productions. However well adapted to a specific environment a type might be, did it lack ever so little of its possibilities in this direction, it was sooner or later relegated to the scrap heap. Some forms, to be sure, persisted in special environments, where they were protected from competition, as in Australia; or where the environment was fairly constant, as in the sea; or in special environments for which they were highly suited; but the whole trend of evolution, with these exceptions, may be summed up by the statement: the general course of evolution has been always from the beginning to the end, in the direc-



tion of increasing adaptability or increasing perfection of irritability. This law may be put by the side of the law for the evolution of universes: all spontaneous change is in the direction of increasing entropy.

It is not by form, by color, by increasing complexity or simplicity, that animals may be classified in the order of their evolutionary appearance. It is by this property of adaptability and this alone. At the summit is man; now consciously attempting to carry on what nature has been unconsciously attempting these millions of years, and to secure mastery of his environment. Below him are the other placental mammals of lower intelligence; beneath them the marsupials, less adaptable than the mammals, because of lower brain power; then the reptiles independent of water, but not of temperature; the amphibia, only partially independent of water, but not of temperature; the teleosts able to live in salt and fresh water; the selachians, most without osmotic control and limited to the sea; the arthropods living on land and sea, but dependent on temperature, food and climate, cramped by an external skeleton, and with the fatal defect of running the alimentary canal through the nervous system, so that for higher brain power, either a new nervous system or a new alimentary canal would be needed; lower still the molluscs and annelids, closely limited to their environments; and last the echinoderms and protozoa. No adaptation or power of the body has been so consistently attacked by natural selection as this; and it is this property which seems to have been the determining factor in the general course of evolution and to have determined the steady development of the psychic powers.

I come now to the second part of my subject, namely, correlation. By the first part I have attempted to show that the selection of variations in adaptability is responsible for at least a part of the steady progress in one direction of many kinds of animals; and explains that unity of progress which has been one of the main causes for assuming orthogenesis. In this second part of the

paper, I hope to show that the development of our knowledge of correlation removes some other difficulties which Darwin had to meet, and probably explains some other facts which have been urged as supporting orthogenesis.

Among the puzzles of evolution has been the steady growth of rudimentary structures which have apparently no function until they are considerably developed. I say *apparently* no function, for the physiologist has learned to be very cautious in saying that any part of the body is without function or use. A few years ago it was quite otherwise and it was supposed that various rudiments, like the appendix, the hypophysis, the pineal gland, the thymus and some other organs were without function; the surgeons were busy explaining how much better we were off without them; and the anti-Darwinian was fond of presenting these things as not consonant with the view of adaptation. At the present time the uselessness of these rudimentary structures is no longer affirmed. We must therefore be very cautious in supposing that any structure we see, no matter how insignificant it may appear, is without importance. Darwin himself felt the great fact of correlation, and his pangene theory was invented, in part, to account for these facts. He would be both astonished and delighted could he know how completely physiology has vindicated his appeal to correlation as the explanation of some difficulties.

Modern physiology has shown that the whole animal organism is correlated by means of internal secretions; that there is but one unit in the body, and that is the whole organism. By the work of Knowlton and Starling we now have the final proof of the correlation of the pancreas and muscles. The correlations between the hard and soft parts of the body are of still greater importance to the paleontologist, for it has been shown that the hard parts are not independently variable, but that they are dependent at every point upon the function of the soft, internal organs. Who would have dreamed that the character of the skin, the hair, the shape of the skull, the in-



telligence itself, the length of the limbs, or the speed of transformation of a tadpole into a frog would be dependent on the thyroid or thymus gland? That the minute parathyroids should be absolutely necessary to the life of an organism thousands of times their weight? Or that the development of the testes, the change of milk teeth to the permanent dentition, the growth of the bones of the extremities, should be dependent on the anterior lobe of that apparently useless rudiment, the hypophysis? Or that the secretion of milk and urine should depend on the posterior lobe of the same organ? Who had the temerity to suggest that the corpus luteum should be influencing the development of the mammary glands? Do we not see, indeed, that most of the characters of the body which have steadily developed from the fishes to man are secondary characters dependent on the anterior development of these ductless glands? Is this fact without significance to the paleontologist in helping him to understand the apparent steady progress in one direction, the appearance of orthogenesis? It will be asked, perhaps, what has caused the steady development of these glands. But the answer is not difficult. They are, in their turn, parts of the mechanism of adaptability which has been consistently selected in evolution. They are concerned not only in the growth of bone, but in the growth of the nervous system, the heat control of the body, the immunity mechanisms, the efficiency of muscles, and are in the chain of reproduction itself. These facts largely remove, in my opinion, the difficulties in understanding how rudimentary organs could be useful.

But not only do these facts remove these difficulties in the way of the selection theory, but they have a no less important bearing on the problem of heredity. They show that there can be no independently variable qualities in the animal body. The body is a unit, and I, at least, can imagine no part of it which can vary without influencing other parts. Correlations are everywhere. Pigment is often cited as a unit character, but how can it

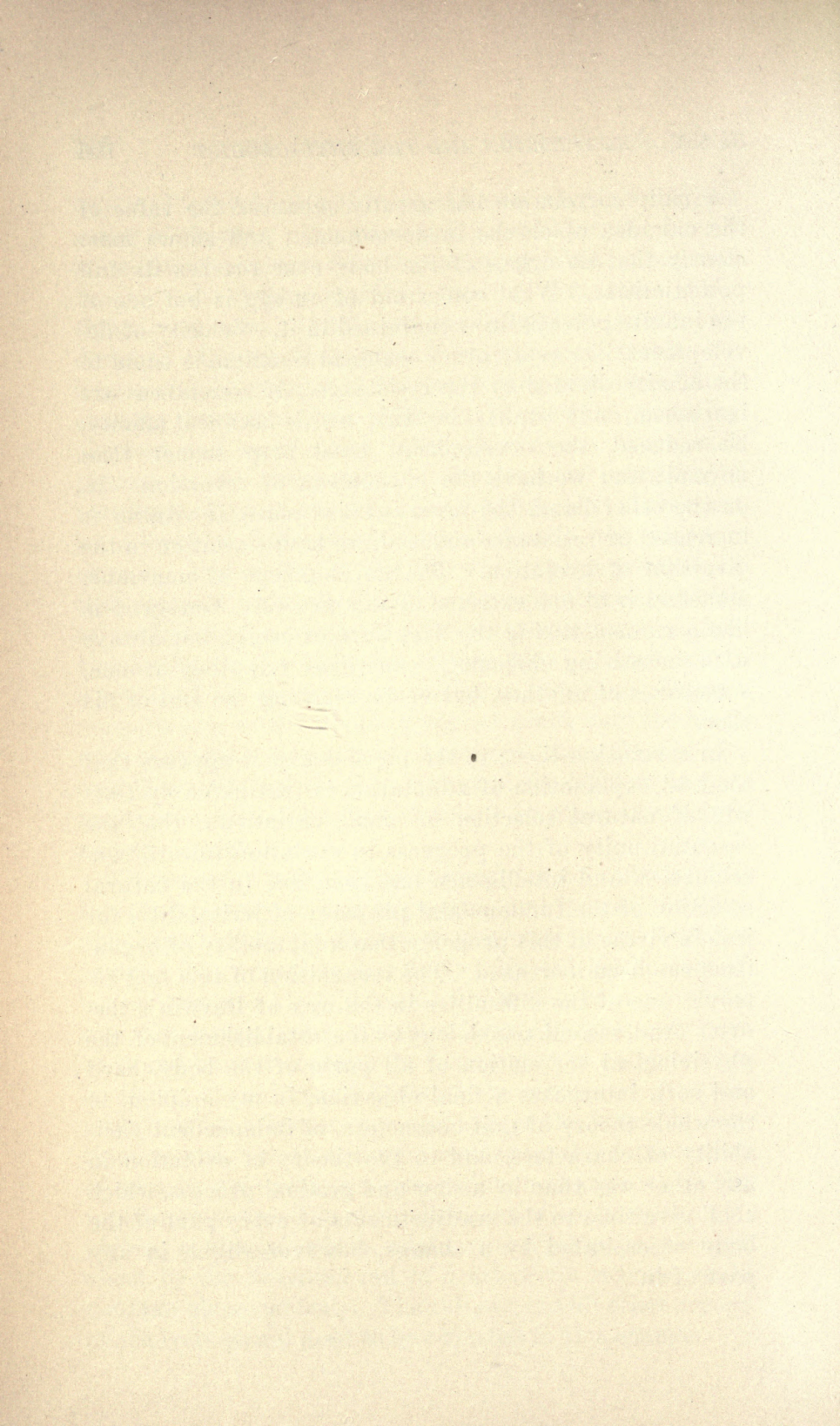
be so? Pigment is itself the result of a long and complex series of changes. If a given cell produces no pigment it is perfectly certain that its other chemical processes are to some degree modified also, so that these other things vary also. If this cell is changed so that it produces no pigment, then since it is the logical result of a long series of changes in the developing organism, those changes must have been different in animals producing pigment and no pigment. But this means, since each process in the early stage of development influences a multitude of processes in the final change, that there must be a host of differences correlated with the pigment change. As a matter of fact, Darwin long ago pointed out that pigment production was apparently correlated with other factors; particularly with vital resistance, a fact repeatedly mentioned to the writer, also by Whitman as a result of his experiments in pigeon breeding. Darwin cites the case of the Virginia pigs of which only the black ones could eat a poisonous root without losing their hoofs; and Whitman told me that always birds deficient in pigment were also somewhat deficient in other characters and were weaker.

The essential unity of the organism is not only fatal to the whole theory of unit characters, but it is an insuperable objection to the theory that evolution has been by jumps. The organism is a finely adjusted mechanism of a very complex kind; it seems impossible to a physiologist that one can cause a sudden large change in any part of it and have it continue to function; it is as incredible as if one should remove one of the wheels of a watch, replace it by a larger one, and expect the watch to continue to run. Such a simple matter as the replacement of urea by uric acid as an excretion, a change which the reptiles introduced in their differentiation from the amphibia, a change which might conceivably be brought about by the dropping out of a uricolytic enzyme, could not take place suddenly. The kidneys and all other organs of the body would need to be adjusted to this change.



Finally correlation has greatly enhanced the value of the old idea of checks in development and shows most clearly that no organ of the body ever reaches its full potentialities. What comes out of an egg is but one of the infinite potentialities contained in it. Velocity of development, like every other chemical reaction, is equal to the affinity divided by the resistance. If resistances are increased, or if vitality, in other words chemical affinity, be reduced, the development must stop sooner than normal; and we have the phenomena of reversion. If, on the other hand, the reverse takes place, if vitality is increased or resistance reduced, we have variation in the direction of evolution. The development of nonviable monsters is at one extreme of this process. Ontogeny is like a runner, taking the first hurdles easily, but always with increasing difficulty, sometimes tripping at one, sometimes at another, but never reaching the end of his race.

In conclusion then: to the physiologist it appears that the best explanation of adaptation is that given by Darwin of natural selection of small variations; that the essential unity of the progress in evolution toward consciousness and intelligence has been due to the natural selection of the fundamental property of irritability, for it is in virtue of this property that adaptability of organisms has been increased. The recognition of this fact removes one of the difficulties in the way of Darwin's theory. And, second, physiology by the establishment of the physiological correlation of all parts of the body, hard and soft, interposes a final objection, in my opinion, to the whole theory of unit characters, of independent variability of characters, and to the theory of evolution in any other way than by a slow and gradual process, which shall give time to the readjustments of every part of the body necessitated by a change, however slight, in any part of it.







# A METHOD OF DETERMINING "a" OF VAN DER WAALS' EQUATION FROM THE SURFACE TENSION

BY ALBERT P. MATHEWS.

Uncertainty still exists as to the correct value of van der Waals' constant "a," which expresses the cohesive pressure of a fluid.

It is well known that if "a" and "b" are both supposed to be constant, the equation of state may be solved and the relationships obtained:  $a = 3P_c V_c^2$ ;  $V_c = 3b$ ;  $P_c = a/27b^2$ ; and  $T_c = 8a/27bR$ . As this solution depends on the erroneous assumption of the constancy of "b," there is no certainty that any of these expressions is correct; and as a matter of fact, only one of them, *i. e.*,  $P_c = a/27b^2$ , happens to approximate very closely to a correct value. Were these relationships true, the calculation of "a" and "b" from the critical data would be very simple; but as they are not true, except for unknown values of "b," it is necessary to find some other means of computing "a," which does not require a knowledge of the real molecular volume, or "b."

While the formula usually employed for the calculation of "a," *i. e.*,  $a = 27V_c^2/64 \times 273^2 \times P_c$ , gives a value at least approximately correct for non-associating substances of medium molecular complexity, it is still uncertain whether the value thus obtained is correct for very simple substances such as hydrogen, or for very complex substances such as diphenyl methane. The ratio 27/64 can only be justified theoretically if "b" were constant and it is probable that this ratio, 27/64, is not constant, but diminishes as molecular compressibility increases. "b<sub>c</sub>" may not always be the same fraction of  $V_c$ , for it is possible that this fraction also varies with the compressibility of the molecule. All other formulas for "a" are also more or less unsatisfactory. Thus in the formula  $a = 6.28V_c^2P_c$ , the coefficient is not the same for all substances. In the formula  $a = 27b_c^2P_c$  a knowledge



of  $b_c$  is required. The calculation of "a" from the latent heat of vaporization by the formula  $(L - E)/(d_1 - D_v) = a/\text{Mol.wt.}$ , where  $L$  is the total latent heat and  $E$  the part consumed in doing external work, is rendered uncertain by the fact that there is still another part of the heat consumed by the expansion of the molecules from the size they are in the liquid to the size they are in the vapor, and this correction is unknown.

The following method of the <sup>putation</sup>consumption of "a" from the surface tension is new, so far as I can find, although it is an application of the very first method used to obtain some idea of the amount of the cohesive pressure of a liquid; it does not involve the value "b;" and it is of interest that the results obtained from it are, on the whole, closely similar to those given by the usual formula. It is, I believe, more trustworthy than the formula usually employed. It is a method proposed by the great English philosopher, Thomas Young, in his epoch-making work on Cohesion. In that work, by an insight little short of marvelous, he came to the conclusion that  $S$ , the surface tension, was equalled to one-third the total cohesive pressure, multiplied into the radius of action of the cohesive attraction, or  $S = rK/3$ .

Since "a" varies with the volume of the gas taken under standard conditions of temperature and pressure, it would be, in many ways, convenient to have a value which was characteristic of the molecules of each substance and which would be independent of the volume of gas or liquid, or the temperature ~~of~~ pressure to which it was subjected. Such a value is very easily obtained by putting "a" equal to  $N^2 M^2 K$ , where  $N$  is the number of molecules in the volume  $V$ ; and writing  $V^2$  as  $Nv^2$ , where small  $v$  is the volume at the disposal of a single molecule. By dividing by  $N^2$  we have then  $a/V^2 = M^2 K/v^2$ . We may call  $M$  the mass of cohesion of a molecule, and  $K$  is a constant of proportion. This same value may be obtained directly by supposing that molecules attract each other inversely as the fourth power of the distance between their centers, directly as the product of their cohesive masses

$N^2 \times V \times N^2 \times V$

and that each molecule attracts only the six surrounding molecules. In another paper I shall show that the value  $M^2K$  is proportional to the two-thirds power of the product of the molecular weight and the number of valences in the molecule. In this paper I wish to show how  $M^2K$  may be derived from the surface tension. The computations are made in absolute units.

The formula,  $S = rK/3$ , states that the surface tension of a liquid is a function of the cohesive pressure of the liquid alone. It is clear, then, that this formula can hold only at very low temperatures, since only at such temperatures can the cohesive pressure in the vapor be neglected. The surface tension, strictly speaking, can not represent the cohesive pressure of the liquid alone, since it is in the very nature of things an expression of the difference in cohesive energy of the liquid and vapor. I shall, then, take the formula as holding at absolute zero, since, if we are going to a temperature in which the vapor may be entirely neglected, it is more convenient to go to the end.

The radius of action of the cohesive attraction, or  $r$ , has been found, both by calculation and by direct measurement, to be, at higher temperatures, very nearly equal to the distance between the molecular centers; and at absolute zero, with the molecules in contact, we may safely assume that " $r$ " is equal to  $v^{1/3}$ .  $K$  is Laplace's constant and is equal to  $a/V^2$ , or  $M^2K/v^2$ . The formula becomes then:  $S = v_o^{1/3}M^2K/3v_o^2 = M^2K/3v_o^{5/3}$ . By multiplying both sides of the equation by  $v_o^{2/3}$  we have,  $Sv_o^{2/3} = M^2K/3v_o$ ;  $v_o$ , the volume at the disposal of one molecule at absolute zero, is, for substances of medium complexity such as ether, very nearly equal to  $v_c$ , the volume of a molecule at the critical temperature, divided by 4. For simpler substances, such as  $O_2$  or  $CO_2$ , the volume  $v_o$  is equal to  $V_c/3.63$ ; and for more complex substances, such as octane, it is  $v_c/4.04$ , or for some even  $v_c/4.10$ . The volume of a molecule is obtained by dividing the volume of a gram mol by  $6.21 \times 10^{23}$ , which is the most probable number of molecules in a gram mol. There is a



difference of opinion as to the value of the coefficient which is given as  $1/3$  by Young,<sup>1</sup> but as  $3/20$  by Rayleigh.<sup>2</sup> As will be shown presently, Young's value is to be preferred.

The value of  $Sv_o^{2/3}$ , the molecular surface tension energy at absolute zero, may be obtained from Eötvös<sup>3</sup> and Ramsay and Shields<sup>4</sup> rule,  $S(mv)^{2/3} = 2.12$  (or  $2.19$ )( $T_c - T - 6$ ).  $S(mv)^{2/3}$  divided by  $N^{2/3}$ , where  $N$  is the number of molecules in one gram mol, equals  $Sv_o^{2/3}$ . Hence  $Sv_o^{2/3} = 2.19(T_c - T - 6)/N^{2/3}$ ; and at absolute zero, where  $T$  is zero,  $Sv_o^{2/3} = 2.19(T_c - 6)/N^{2/3} = 3.015 \times 10^{-16}(T_c - 6)$  ergs. It is assumed that the law holds clear to absolute zero.

I have used the coefficient  $2.19$  because I thought it more typical of a non-associating substance, and it is nearer to the value of Eötvös. However, this has given me slightly higher values for "a" than those generally computed, as may be seen in the table. The agreement with other values of "a" seemed on the whole better with this coefficient. It varies slightly with different substances in any case being nearly  $2$ , for some gases like oxygen, and as high as  $2.3$  for very complex substances.  $2.19$  comes close to the mean.

We have then  $Sv_o^{2/3} = M^2K/3v_o = 3.015 \times 10^{-16}(T_c - 6)$  ergs. So  $M^2K = 9.045 \times 10^{-16}(T_c - 6)v_o$ .

The value  $6^\circ$  subtracted from the critical temperature was established for substances having a critical temperature of about  $400^\circ$ – $480^\circ$  Abs. It would be better perhaps to make it  $1/80$ th of  $T_c$ . The formula corrected thus would be  $M^2K = 9.045 \times 10^{-16}T_c v_o 79/80 = 8.932 \times 10^{-16}T_c v_o$ . As this last correction is uncertain, however, I have uniformly subtracted  $6^\circ$  from the critical temperature, unless it is specifically stated to the contrary.

<sup>1</sup> Young: "An Essay on the Cohesion of Fluids," Philosophical Transactions, 1805. (Collected works, edited by G. Peacock, Vol. I, 1855, p. 418, London.)

<sup>2</sup> Rayleigh: Article on "Capillarity," Encyclopaedia Britannica, xi edition.

<sup>3</sup> Eötvös: Wied. Ann., 27, 458 (1886).

<sup>4</sup> Ramsay and Shields: Zeit. phys. Chem., 12, 433 (1893).

As already mentioned there is an uncertainty whether the coefficient should be  $\frac{1}{3}$ , as found by Young, or  $\frac{3}{20}$ , as found by Rayleigh. As I was unable to decide which of the values of the coefficient was preferable, I tried them both, and Young's value gives a consistent result. Rayleigh's gives an impossible result, the cohesive pressure computed by it being more than double what it ought to be, so that if it be substituted in van der Waals' equation,  $b_c$  must be taken very large and at what are impossible values. For example,  $b_c$  must be  $0.748V_c$ , or nearly three-fourths of the critical volume, if the coefficient  $\frac{3}{20}$  is used. Such a value for " $b$ " is impossible if the atoms retain their uniform size in the molecules, or if they change very little, since the intra-molecular cohesion is so much greater than the intermolecular that the greater part of the gain in volume in coming from zero to the critical temperature must be in the spaces between the molecules, rather than within them. If the atoms are incompressible and make up one-fourth of the total volume, then three-fourths of the critical volume will be free space. If  $b_c = 0.75V_c$  and the atomic volume is  $0.25V_c$ , then free space within the molecule will be  $0.5V_c$ , leaving only a total of  $0.25V_c$  for the inter-molecular space. In other words, the space within the molecules would have enlarged more in passing from zero to the critical temperature than the space between the molecules. Such a result is impossible. With Young's formula, however,  $b_c = 0.5V_c$  approximately; if the atomic volume is  $0.25V_c$ , then the free space within the molecules would be  $0.25V_c$  and between the molecules,  $0.5V_c$ , which is a more probable result.

The following values of  $M^2K$  were obtained by the formula,  $M^2K = v_c \times 9.045 \times 10^{-16}(T_c - 6)/4$ :

Substance	$\log_{10} M^2K$
Pentane	—35.71919
Isopentane	—35.70663
Methyl acetate	—35.61791
Ether	—35.67593



With the values thus found for four of the most carefully investigated non-associating substances, the value of the real molecular volume, or  $b_c$ , at the critical temperature was computed by substituting in van der Waals' equation, with the following result:

Substance	$V_c$	$b_c$	$V_c/b_c$
Ether	282.23	135.81	2.078
Pentane	309.94	149.44	2.074
Methyl acetate	227.55	109.09	2.086
Isopentane	307.30	148.30	2.072

By substituting the values of  $M^2K$  derived in the foregoing manner from the surface tension,  $b_c$  turns out to be the same fraction of  $V_c$  for the three best investigated, normal substances, ether, pentane and iso-pentane, a result anticipated from van der Waals' reduced, characteristic equation.  $V_c$  is, therefore,  $2.074b_c$ . The value thus obtained for  $b_c$ , namely  $V_c/2.074$ , is very close to that calculated by van der Waals<sup>1</sup> from a value of "a" obtained from the coefficient of compressibility. He thus obtained the value  $V_c = 2.03b_c$ . Other estimates of  $b_c$  made by him bring it about 2.08. This value of  $b_c$ , moreover, is inherently probable, the value of  $b_c$  necessarily being close to  $V_c/2$ , and presumably a little less than this.

We may now, with this value of  $b_c$  fixed, calculate by substitution in van der Waals' equation the value of the coefficients in the formulas given on page 154 in place of those derived when "a" and "b" were considered constant.

The following relationships were obtained:<sup>2</sup>

- 1  $a/P_c = 6.284V_c^2$ ; in place of  $a/P_c = 3V_c^2$ .
- 2  $V_c = 2.074b_c$ ; in place of  $V_c = 3b_c$ .
- 3  $P_c = a/27.02b_c^2$ ; in place of  $P_c = a/27b_c^2$ .
- 4  $T_c = 7.769a/27.02Rb_c$ ; in place of  $T_c = 8a/27Rb_c$ .
- 5  $b_c + RT_c/P_c = 4.243V_c$ ; in place of  $b_c + RT_c/P_c = 3V_c$ .
- 6  $P_cV_c/T_c = R \cdot 2.074/7.769 = 21.92$ ; in place of  $P_cV_c/T_c = 3R/8 = 30.80$ .
- 7  $RT_c/P_cV_c = 3.75$ .

<sup>1</sup> Van der Waals: Proc. Roy. Acad. Sci., Amsterdam, 3, 583 (1901).

<sup>2</sup> Compare with van der Waals: Proc. Roy. Acad. Sci., Amsterdam, 13,

TABLE I

Values of  $M^2K$ , as calculated by different formulas, expressed in absolute units. (1)  $M^2K = V_c T_c 3.594 \times 10^{-40}$ ; (2)  $M^2K = P_c V_c^2 1.6296 \times 10^{-47}$ ; (3)  $M^2K = 9.045 \times 10^{-16} (T_c - 6)v_0$ ; (4)  $M^2K = 27T_c^2/64 \times (273)^2 P_c N^2$ .  
Critical data of all substances those of Young.

Substances	Formulas			
	1	2	3	4
SnCl <sub>4</sub>	$7.476 \times 10^{-35}$	$7.538 \times 10^{-35}$	$7.499 \times 10^{-35}$	$7.083 \times 10^{-35}$
Diisobutyl	$9.522 \times 10^{-35}$	$9.411 \times 10^{-35}$	$9.541 \times 10^{-35}$	$9.202 \times 10^{-35}$
Hexamethylene	$6.105 \times 10^{-35}$	$6.201 \times 10^{-35}$	$6.118 \times 10^{-35}$	$5.742 \times 10^{-35}$
Diisopropyl	$6.415 \times 10^{-35}$	$6.457 \times 10^{-35}$	$6.421 \times 10^{-35}$	$6.089 \times 10^{-35}$
Isopentane	$5.090 \times 10^{-35}$	$5.132 \times 10^{-35}$	$5.089 \times 10^{-35}$	$4.821 \times 10^{-35}$
Methyl formate	$2.992 \times 10^{-35}$	$2.893 \times 10^{-35}$	$2.993 \times 10^{-35}$	$2.992 \times 10^{-35}$
Carbon tetrachloride	$5.514 \times 10^{-35}$	$5.650 \times 10^{-35}$	$5.526 \times 10^{-35}$	$5.142 \times 10^{-35}$
Fluorbenzene	$5.453 \times 10^{-35}$	$5.416 \times 10^{-35}$	$5.465 \times 10^{-35}$	$5.245 \times 10^{-35}$
Chlorbenzene	$6.993 \times 10^{-35}$	$6.981 \times 10^{-35}$	$7.017 \times 10^{-35}$	$6.692 \times 10^{-35}$
Brombenzene	$7.791 \times 10^{-35}$	$7.712 \times 10^{-35}$	$7.810 \times 10^{-35}$	$7.522 \times 10^{-35}$
Iodobenzene	$9.093 \times 10^{-35}$	$9.070 \times 10^{-35}$	$9.135 \times 10^{-35}$	$8.711 \times 10^{-35}$
Benzene	$5.170 \times 10^{-35}$	$5.188 \times 10^{-35}$	$5.181 \times 10^{-35}$	$4.921 \times 10^{-35}$
Methyl butyrate	$6.770 \times 10^{-35}$	$6.535 \times 10^{-35}$	$6.784 \times 10^{-35}$	$6.698 \times 10^{-35}$
Methyl isobutyrate	$6.580 \times 10^{-35}$	$6.413 \times 10^{-35}$	$6.591 \times 10^{-35}$	$6.448 \times 10^{-35}$
Hexane (normal)	$6.697 \times 10^{-35}$	$6.583 \times 10^{-35}$	$6.704 \times 10^{-35}$	$6.508 \times 10^{-35}$
Heptane (normal)	$8.289 \times 10^{-35}$	$8.099 \times 10^{-35}$	$8.303 \times 10^{-35}$	$8.103 \times 10^{-35}$
Octane (normal)	$10.025 \times 10^{-35}$	$9.766 \times 10^{-35}$	$10.050 \times 10^{-35}$	$9.826 \times 10^{-35}$
Ether (ethyl)	$4.736 \times 10^{-35}$	$4.685 \times 10^{-35}$	$4.797 \times 10^{-35}$	$4.574 \times 10^{-35}$
Pentane	$5.238 \times 10^{-35}$	$5.238 \times 10^{-35}$	$5.239 \times 10^{-35}$	$5.004 \times 10^{-35}$
Ethyl formate	$4.183 \times 10^{-35}$	$4.047 \times 10^{-35}$	$4.188 \times 10^{-35}$	$4.130 \times 10^{-35}$
Methyl acetate	$4.144 \times 10^{-35}$	$3.961 \times 10^{-35}$	$4.149 \times 10^{-35}$	$4.142 \times 10^{-35}$
Propyl formate	$5.500 \times 10^{-35}$	$5.357 \times 10^{-35}$	$5.510 \times 10^{-35}$	$5.395 \times 10^{-35}$
Ethyl acetate	$5.377 \times 10^{-35}$	$5.131 \times 10^{-35}$	$5.397 \times 10^{-35}$	$5.383 \times 10^{-35}$
Methyl propionate	$5.371 \times 10^{-35}$	$5.177 \times 10^{-35}$	$5.377 \times 10^{-35}$	$5.322 \times 10^{-35}$
Propyl acetate	$6.810 \times 10^{-35}$	$6.521 \times 10^{-35}$	$6.823 \times 10^{-35}$	$6.791 \times 10^{-35}$
Ethyl propionate	$6.750 \times 10^{-35}$	$6.483 \times 10^{-35}$	$6.772 \times 10^{-35}$	$6.711 \times 10^{-35}$
Propyl alcohol	$4.234 \times 10^{-35}$	$3.989 \times 10^{-35}$	$4.241 \times 10^{-35}$	$4.293 \times 10^{-35}$



The value of 21.92 thus obtained for  $P_c V_c / T_c$  corresponds with the value determined from Young's very careful observations on the critical data of some non-associating substances.

The values obtained for  $M^2K$  by the use of the foregoing formulas, the surface tension formula, and the ordinary formula, when applied to the critical data of the substances so carefully investigated by Young<sup>1</sup> are given in Table I.

The value of "a" in atmospheres for 1 cc of a gas under standard conditions of pressure and temperature may be obtained from any of the values in this table by multiplying by  $7.573 \times 10^{32}$ .

A comparison of columns 3 and 4 of Table I will show that the values obtained by the surface tension formula, while on the whole closely similar to those obtained by the ordinary formula given in column 4, differ, nevertheless, in some instances quite markedly from them. The values of column 3, on the other hand, are almost identical with those of column 1. Owing to the fact that there is less uncertainty about the factors used in computing "a" from the surface tension than from the usual formula, I believe the surface tension values are to be preferred. If the coefficient in the surface tension formula had been taken as 2.12 instead of 2.19, all the values of column 3 would need to be reduced proportionally, and the values in columns 1 and 2 would also be lower. That the values of "a" computed in this way from the surface tension are the more accurate is indicated also by the fact that these values exhibit most clearly and with fewest exceptions the relation of cohesion to molecular weight and the number of valences in the molecule, as is set forth in a subsequent paper.

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<sup>1</sup> Young: "The Vapor Pressures, Specific Volumes, Heats of Vaporization and Critical Constants of 30 Pure Substances," Proc. Roy. Dublin Soc., 12, 374-443 (1910).





# THE RELATION OF THE VALUE "a" OF VAN DER WAALS' EQUATION TO THE MOLECULAR WEIGHT AND THE NUMBER OF VALENCES OF THE MOLECULE

BY ALBERT P. MATHEWS

The discovery of the properties of the molecule upon which cohesion depends is a matter of great interest. I have found that "a" of van der Waals' equation is equal to a constant multiplied into the square of the cube root of the product of the molecular weight by the number of valences in the molecule. This enables a calculation of the valence number of a molecule from the critical constants; and on the other hand a calculation of "a" from the valence and molecular weight. Some interesting facts have been discovered relative to the valence of the halogens, of the argon group and some other elements by the application of this rule.

If the value  $a/V^2$  of van der Waals' equation, which represents the internal, or cohesive, pressure per unit surface, be divided in both the numerator and denominator by  $N^2$ ,  $N$  being the number of molecules in the volume  $V$ , we obtain a value for "a" which may be called the molecular cohesive pressure and which is independent of the volume. If "a" be represented by the expression  $N^2M^2K$ , in which  $M$  is the mass of cohesion of a molecule and  $K$  a constant, then since  $V^2$  is equal to  $N^2v^2$ ,  $v$  being the volume at the disposal of a single molecule,  $a/V_2 = N^2M^2K/N^2v^2 = M^2K/v^2$ . This value  $M^2K$  may be computed from "a" by dividing the latter by  $N^2$ .  $N$ , the number of molecules in a cc. of gas under standard conditions, is equal to  $2.77 \times 10^{19}$ . In the computations which follow I have taken the value of "a" in dynes instead of atmospheres and wherever possible I have used the value of  $M^2K$  computed by the surface tension formula described in a previous paper.<sup>1</sup>

<sup>1</sup> Mathews: Jour. Phys. Chem., 17, 154 (1913).

Tables I and II bring out the relationship that  $M$  is some function of the molecular weight and the number of valences in the molecule, or  $M^2K = f(\text{Wt})(\text{Valence})$ . The relationship to molecular weight appears if we compare compounds of the same valence number as shown in Table I.

TABLE I

Substance	Mol. weight	Valences	$M^2K \times 10^{35}$
$\text{CO}_2$	44.0	8	1.216
$\text{CCl}_4$	153.8	8 <sup>1</sup>	5.526
$\text{GeCl}_5$	214.3	8	6.274
$\text{SnCl}_4$	260.8	8	7.499
$\text{C}_6\text{H}_6$	78.0	30	5.181
$\text{C}_6\text{H}_5\text{F}$	96.0	30	5.465
$\text{C}_6\text{H}_5\text{Cl}$	112.45	30	7.017
$\text{C}_6\text{H}_5\text{Br}$	157.0	30	7.810(?)
$\text{C}_6\text{H}_5\text{I}$	204.0	30	9.135(?)
Ether	74.0	28	4.797
Methyl propionate	88.0	28	5.377
Ethyl acetate	88.0	28	5.397

It is clear from Table I that the factor  $M^2K$  increases steadily with the molecular weight.

The importance of valence is shown in Table II. A heavier compound, if it have fewer valences, may have a lower mass of cohesion than a lighter substance.

TABLE II

Substance	Mol. weight	Valences	$M^2K \times 10^{35}$
$\text{C}_6\text{H}_5\text{I}$	204.0	30	9.135
$\text{SnCl}_4$	260.8	8	7.499
$\text{C}_6\text{H}_5\text{Br}$	157.0	30	7.810
$\text{CCl}_4$	153.8	8	5.526
Iso-pentane	72.0	32	5.089
Ether	74.0	28	4.797
Ethyl formate	74.0	22	4.188

<sup>1</sup> The valence of the halogens in this and the next table is, for simplicity, taken as unity. Their true valence is discussed farther on.



It might also be imagined that the mass of cohesion was a function of the number of atoms in the molecule, rather than the number of valences, but a trial of this possibility showed that this was not the case.

After many trials in which the mass of cohesion, or the factor  $M^2K$ , was supposed to be a function of the first power, the square, the square root, or the square of the cube root of the product of the number of valences and the molecular weight, it was found that only the last supposition yielded a consistent result. In all the other guesses, the proportionality factor "*C*" was far from being constant. Hence we have:  $M^2K = C(Wt \times Val.)^{2/3}$ .

With the values of  $M^2K$  computed by the surface tension formula it was found that the quotient  $M^2K/(Wt. \times Val.)^{2/3}$  was indeed as constant as could be expected and *C* had a mean value of  $2.98 \times 10^{-37}$ , when  $M^2K$  is the square of the mass of cohesion of one molecule multiplied by *K* and expressed in absolute units. If "*a*" of van der Waals' equation is taken for 1 cc. of gas under standard conditions and expressed in atmospheres, the proportionality factor *C* would be  $2.2564 \times 10^{-4}$ . This gives values for "*a*" a little higher than those generally accepted owing to my having used the coefficient 2.19 instead of 2.12 in computing  $M^2K$  from the surface tension. The computation of "*a*" for ether by the formula  $2.2564 \times 10^{-4} (Weight \times Valences)^{2/3}$  gives the value 0.03669, whereas the value from the usual formula of van der Waals is 0.03473; the coefficient 2.12 would have given the value 0.03550. The fact that these values are throughout proportionally somewhat higher than those usually assumed does not affect the constancy of *C*.

Table III shows that the quotient  $M^2K/(Wt. Val.)^{2/3}$  really equals a constant when  $M^2K$  is computed for the compounds of carbon, hydrogen and oxygen of which the critical data have been so carefully determined by Young. For the elements of these compounds there is no doubt of the valence number. Carbon is always quadrivalent, hydrogen univalent; and oxygen has been taken as bivalent.

TABLE III  
 $C = M^2K / (Wt. Val.)^2$

Substance	Formula	Log <sub>10</sub> M <sup>2</sup> K	Mol. wt.	No. of valences	$C \times 10^{37}$	Remarks
1 Pentane	C <sub>5</sub> H <sub>12</sub>	-35.71925	72	32	3.00	
2 Isopentane	C <sub>5</sub> H <sub>12</sub>	-35.70663	72	32	2.92	
3 Ether	C <sub>4</sub> H <sub>10</sub> O	-35.68097	74	28	2.95	
4 Et formate	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	-35.62201	74	22	3.03	
5 Me acetate	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	-35.61794	74	22	3.00	
6 Me formate	C <sub>3</sub> H <sub>4</sub> O <sub>2</sub>	-35.47611	60	16	3.08	
7 Benzene	C <sub>6</sub> H <sub>6</sub>	-35.71441	78	30	2.94	
8 Hexamethylene	C <sub>6</sub> H <sub>12</sub>	-35.78661	84	36	2.93	
9 Diisopropyl	C <sub>6</sub> H <sub>14</sub>	-35.80760	86	38	2.92	
10 Hexane	C <sub>6</sub> H <sub>14</sub>	-35.82633	86	38	3.04	
11 Et acetate	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	-35.73215	88	28	2.96	
12 Prop formate	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	-35.74939	88	28	3.01	
13 Me propionate	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	-35.73954	88	28	2.95	
14 Fluorobenzene	C <sub>6</sub> H <sub>5</sub> Fl	-35.75295	96	30	2.80	Fl = 1; V <sub>c</sub> /V <sub>o</sub> = 3.86
15 Heptane	C <sub>7</sub> H <sub>16</sub>	-35.91924	100	44	3.09	
16 Me butyrate	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	-35.83149	102	34	2.96	
17 Me isobutyrate	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	-35.81895	102	34	2.88	
18 Chlorobenzene	C <sub>6</sub> H <sub>5</sub> Cl	-35.84615	112.45	32	2.99	Cl = 3
19 Octane	C <sub>8</sub> H <sub>18</sub>	-34.00100	114	50	3.15	
20 Diisobutyl	C <sub>8</sub> H <sub>18</sub>	-35.97959	114	50	2.99	
21 Prop acetate	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	-35.83398	102	34	2.98	
22 Et propionate	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	-35.83972	102	34	2.96	
23 Brombenzene	C <sub>6</sub> H <sub>5</sub> Br	-35.89265	157	30	2.78	T <sub>c</sub> and V <sub>c</sub> estimated
24 Iodobenzene	C <sub>6</sub> H <sub>5</sub> I	-35.96071	204	30	2.73	T <sub>c</sub> and V <sub>c</sub> estimated
25 Carbon tetrachloride	CCl <sub>4</sub>	-35.74247	153.8	16	3.03	Cl = 3
26 Stannic chloride	SnCl <sub>4</sub>	-35.87500	260.8	16	2.89	Cl = 3

Mean (omitting C<sub>8</sub>H<sub>5</sub>I and C<sub>8</sub>H<sub>5</sub>Br), 2.98



It will be seen in Table III that  $C$  has a mean value of  $2.98 \times 10^{-37}$  for the twenty-six non-associating, or nearly non-associating, substances studied by Young. The greatest deviation from the mean is octane on the one side, which deviates a little more than four percent; and on the other, fluorobenzene, which deviates 6 percent, methyl isobutyrate deviating a little over 3 percent, and brom- and iodobenzene. The deviation of the last two substances, about 7 percent, may, I think, be disregarded since the critical temperature and pressure were estimated and not directly determined; the critical data are therefore less certain. The other twenty-one substances do not deviate more than 3 percent from the mean. The cause of the deviation of fluorobenzene is uncertain, but there is a regularity in the other deviations which suggests that the size, shape, or compressibility of the molecule may play a slight rôle in determining the critical data, or the coefficients of our formulas. For it appears that  $M^2K$ , and hence  $C$ , is always lower in the iso-compounds than in the corresponding normal compounds. Diisobutyl has  $C$  of the mean value, whereas normal octane has a high value for  $C$ ; a similar relationship exists between diisopropyl and hexane; and between iso- and normal pentane. Some of this difference would disappear if, instead of taking  $v_o$  equal to  $v_c/4$ , I had used the accurate ratio computed from the law of rectilinear diameter. Thus  $V_o$  of isopentane is only  $V_c/3.92$ ; while that of pentane is  $V_c/3.96$  and octane is  $V_c/4.04$ . The substitution of these values for  $V_o$  in computing  $M^2K$  would have made  $C$  of isopentane 2.98; of pentane, 3.03. The difference is not entirely due then to this factor. It seems more probable to me that  $b_c$  is not always exactly the same fraction of  $V_c$  and that consequently the coefficients of the formulas used in computing  $M^2K$  are not exactly the same in all substances.

Notwithstanding these slight deviations, the constancy of  $C$  is certainly remarkably good and shows beyond question, I think, that a close connection exists between molecular cohesion and the molecular weight and number of valences in the molecule.

TABLE IV

Substance	Formula	Log <sub>10</sub> M <sup>2</sup> K	Mol. wt.	No. of valences	C × 10 <sup>37</sup>	Critical data used in computing M <sup>2</sup> K
1 Ethylene	C <sub>2</sub> H <sub>4</sub>	-35.15671 <sup>1</sup>	28	12	2.97	d <sub>c</sub> = 0.21; T <sub>c</sub> = 10; P <sub>c</sub> = 51.7
2 Toluene	C <sub>7</sub> H <sub>8</sub>	-35.82459	92	36	3.01	T <sub>c</sub> = 320.6; P <sub>c</sub> = 41.6
3 Cymol	C <sub>10</sub> H <sub>14</sub>	-34.06800	134	54	3.13	T <sub>c</sub> = 378.6; P <sub>c</sub> = 28.6
4 Decane	C <sub>10</sub> H <sub>22</sub>	-34.12922	142	62	3.16	T <sub>c</sub> = 330.4; P <sub>c</sub> = 21.3
5 Diethylamine	C <sub>4</sub> H <sub>11</sub> N	-35.68002	73	30	2.84	T <sub>c</sub> = 222; P <sub>c</sub> = 40
6 Dimethylamine	C <sub>2</sub> H <sub>7</sub> N	-35.42717	45	18	3.08	T <sub>c</sub> = 163; P <sub>c</sub> = 56
7 Ethane	C <sub>2</sub> H <sub>6</sub>	-35.21866	30	14	2.95	T <sub>c</sub> = 35; P <sub>c</sub> = 45.2
8 Acetylene	C <sub>2</sub> H <sub>2</sub>	-35.08564	26	10	2.99	T <sub>c</sub> = 35.5; P <sub>c</sub> = 61.6
9 Propane	C <sub>3</sub> H <sub>8</sub>	-35.39037	44	20	2.68	T <sub>c</sub> = 97; P <sub>c</sub> = 44
10 Propyl amine	C <sub>3</sub> H <sub>9</sub> N	-35.58048	59	24	3.02	T <sub>c</sub> = 218; P <sub>c</sub> = 50
11 Propyl benzol	C <sub>9</sub> H <sub>12</sub>	-35.99865	120.0	48	3.10	T <sub>c</sub> = 365.6; P <sub>c</sub> = 32.3
12 Propyl chloride	C <sub>3</sub> H <sub>7</sub> Cl	-35.59465	78.45	20	2.91	T <sub>c</sub> = 221; P <sub>c</sub> = 49; Cl = 1
13 Pseudo-cumol	C <sub>9</sub> H <sub>12</sub>	-34.00767	120	48	3.17	T <sub>c</sub> = 381.2; P <sub>c</sub> = 33.2
14 Me valerate	C <sub>8</sub> H <sub>12</sub> O <sub>2</sub>	-35.92785	116	40	3.05	T <sub>c</sub> = 293.7; P <sub>c</sub> = 31.5; d <sub>c</sub> = 0.279
15 Phenetol	C <sub>8</sub> H <sub>10</sub> O	-35.97509	122	44	3.08	T <sub>c</sub> = 374; P <sub>c</sub> = 33.8
16 Chloroform	CHCl <sub>3</sub>	-35.61128	119.35	14	2.90	T <sub>c</sub> = 260; P <sub>c</sub> = 54.9; Cl = 3
17 Me chloride	CH <sub>3</sub> Cl	-35.26811	50.45	10	2.93	T <sub>c</sub> = 141.5; P <sub>c</sub> = 73; Cl = 3
18 Me fluoride	CH <sub>3</sub> F	-35.10958	34	8	3.07	T <sub>c</sub> = 44.9; P <sub>c</sub> = 62; Fl = 1
19 Isoamylene	C <sub>3</sub> H <sub>10</sub>	-35.69737	70	30	3.04	T <sub>c</sub> = 191.6; P <sub>c</sub> = 33.9
20 Isobutyl benzene	C <sub>10</sub> H <sub>14</sub>	-34.03059	134	54	2.87	T <sub>c</sub> = 377.1; P <sub>c</sub> = 31.1
21 Isopropyl benzene	C <sub>9</sub> H <sub>12</sub>	-35.99603	120	48	3.08	T <sub>c</sub> = 362.5; P <sub>c</sub> = 32.2

<sup>1</sup> This is a mean value of M<sup>2</sup>K computed by the several different formulas given in my previous paper.



TABLE IV (Continued)

22	Methyl ether	$C_2H_6O$	-35.35126	46	16	2.76	$T_c = 129.6; P_c = 57$
23	Methyl ethyl ether	$C_3H_8O$	-35.52175	60	22	2.76	$T_c = 168.4; P_c = 46.3;$ $d_c = 0.307$
24	Dimethyl-o-toluidine	$C_9H_{13}N$	-34.03389	135	52	2.95	Guye and Mallet "a"
25	Dipropyl amine	$C_6H_{15}N$	-35.88675	101	42	2.94	$T_c = 277; P_c = 31$
26	Germanium tetrachloride	$GeCl_4$	-35.79817	214.3	16	2.76	$T_c = 276.9; P_c = 38; Cl = 3$
27	Anisol	$C_7H_8O$	-35.87207	108	38	2.91	Guye and Mallet "a"
28	Ethyl butyrate	$C_6H_{12}O_2$	-35.88584	108	40	2.90	$T_c = 292.8; P_c = 30.24$
29	Ethyl chloride	$C_2H_5Cl$	-35.49356	64.45	16	3.05	$T_c = 182.6; P_c = 52.6;$ $Cl = 3$
30	Ethylidene chloride	$C_2H_4Cl_2$	-35.63725	98.9	18	2.95	$T_c = 250; P_c = 50; Cl = 3$
31	Xylol	$C_8H_{10}$	-35.93390	106	42	3.17	$T_c = 344; P_c = 35$
32	Triethylamine	$C_6H_{15}N$	-35.87209	101	42	2.84	$T_c = 259; P_c = 30$
33	Trimethylamine	$C_3H_9N$	-35.55859	59	24	2.87	$T_c = 160.5; P_c = 41$
34	Carbon bisulphide	$CS_2$	-35.53733	76	16	3.03	$T_c = 273; P_c = 73; S = 6;$ $V_c = 175.6$
35	Isobutyl acetate	$C_6H_{12}O_2$	-35.91209	116	49	2.94	$T_c = 295.8; P_c = 31.4$
36	Diphenyl	$C_{12}H_{10}$	-34.14224	154	58	3.22	$T_c = 495.6; P_c = 31.8$
37	Diphenyl methane	$C_{13}H_{12}$	-34.18482	166	64	3.17	$T_c = 497; P_c = 28.2$
38	Durene	$C_{10}H_{14}$	-34.07619	134	54	3.19	$T_c = 402.5; P_c = 28.6$
39	Mesitylene	$C_9H_{12}$	-35.96426	120	48	2.87	$T_c = 367.6; P_c = 33.2$
40	Ethyl benzol	$C_8H_{10}$	-35.90224	106	42	2.95	$T_c = 346.4; P_c = 38.1$
41	Isobutyl formate	$C_5H_{10}O_2$	-35.84701	102	34	3.07	$T_c = 278.2; P_c = 38.3;$ $d_c = 0.2879$

Mean value of C 2.98

Table IV is a summary of the values of  $C$  computed for forty-one other substances, of many of which the critical data are not so accurately known as those included in Table III. Since for most of these substances  $d_c$  and  $V_c$  were not given in the Landolt-Börnstein-Meyerhoffer tables, I computed  $V_c$  by the formula  $V_c P_c / T_c = 21.92$  and then  $M^2K$  by the formula  $M^2K = 3.594 \times 10^{-40} V_c T_c$ . The values of  $V_c$  so computed are of course not so accurate. Wherever the critical volume or density was given it was used. In a few cases, which are specified,  $M^2K$  was obtained by dividing the value of " $a$ ," given by Guye and Mallet, by  $N^2$ , *i. e.*,  $(2.77 \times 10^{19})^2$ .

While the values are thus less certain and the deviations somewhat greater, the mean value of all is what it was for the other substances, *i. e.*,  $C = 2.98 \times 10^{-37}$ . The values are surprisingly uniform. It will be noticed that the more complex substances such as diphenyl and diphenyl methane have  $C$  a little high. This may possibly be due to slight association or quasi-association in these substances. With this and one or two other exceptions the uniformity of  $C$  is marked.

All substances known to be associating give a value for  $C$  higher than  $2.98 \times 10^{-37}$  when  $M^2K$  is computed by the same formulas as for normal substances, and the weight and valence used in computing  $C$  are those of the normal non-associated molecule. This result is to be anticipated, since in these substances the molecular weight and valence do not remain the same throughout the temperature interval. The computation of  $M^2K$  from the critical data is uncertain in the case of associating substances for the reason that there is no certainty that the coefficients of the formulas are the same for these substances as for normal substances. Although the figures for  $M^2K$  are thus uncertain, I have nevertheless calculated  $M^2K$  by the usual formula in order to show that these substances deviate in the direction we should expect from the value of  $C$  found in normal substances. The results are given in Table V:



TABLE V

Substance	Formula	Log <sub>10</sub> M <sup>2</sup> K	Normal weight	No. of valences	C × 10 <sup>37</sup>	Remarks
1 Propyl alcohol	C <sub>3</sub> H <sub>8</sub> O	—35.62672	60	22	3.52	M <sup>2</sup> K = V <sub>c</sub> T <sub>c</sub> <sup>3.594</sup> × 10 <sup>-40</sup>
2 Methyl alcohol	CH <sub>3</sub> O	—35.33602	32	10	4.63	M <sup>2</sup> K = V <sub>c</sub> T <sub>c</sub> <sup>3.594</sup> × 10 <sup>-40</sup>
3 Ethyl alcohol	C <sub>2</sub> H <sub>6</sub> O	—35.56793	46	16	4.54	M <sup>2</sup> K = V <sub>c</sub> T <sub>c</sub> <sup>3.594</sup> × 10 <sup>-40</sup>
4 Acetic acid	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	—35.56329	60	16	3.76	M <sup>2</sup> K = V <sub>c</sub> T <sub>c</sub> <sup>3.594</sup> × 10 <sup>-40</sup>
5 Propionitrile	C <sub>3</sub> H <sub>5</sub> N	—35.77583	55	20	5.60	M <sup>2</sup> K = T <sub>c</sub> <sup>2</sup> (3.057 × 10 <sup>9</sup> )/PN <sup>2</sup>
6 Benzonitrile	C <sub>7</sub> H <sub>5</sub> N	—35.96933	103	36	3.87	M <sup>2</sup> K = T <sub>c</sub> <sup>2</sup> (3.057 × 10 <sup>9</sup> )/PN <sup>2</sup>
7 Capronitrile	C <sub>6</sub> H <sub>11</sub> N	—35.97752	97	38	3.98	M <sup>2</sup> K = T <sub>c</sub> <sup>2</sup> (3.057 × 10 <sup>9</sup> )/PN <sup>2</sup>
8 Butyronitrile	C <sub>4</sub> H <sub>7</sub> N	—35.85452	69	26	4.85	
9 Ammonia	NH <sub>3</sub>	—35.04731	17	6	5.11	T <sub>c</sub> = 130; P <sub>c</sub> = 115
10 Hydrogen sulphide	H <sub>2</sub> S	—35.08657	34	4	4.64	T <sub>c</sub> = 100; P <sub>c</sub> = 90
11 Methyl amine	CH <sub>3</sub> N	—35.30293	31	12	3.88	T <sub>c</sub> = 155; P <sub>c</sub> = 72
12 Phosphoretted hydrogen	PH <sub>3</sub>	—35.11711	34	6	3.78	T <sub>c</sub> = 52.8; P <sub>c</sub> = 64
13 Meta cresol	C <sub>7</sub> H <sub>8</sub> O	—35.94056	108	38	3.40	T <sub>c</sub> = 432; P <sub>c</sub> = 45
14 Aniline	C <sub>6</sub> H <sub>7</sub> N	—35.86700	93	34	3.42	T <sub>c</sub> = 425.7; P <sub>c</sub> = 52.35
15 Thiophene	C <sub>4</sub> H <sub>4</sub> S	—35.76101	84	22	3.83	T <sub>c</sub> = 317.3; P <sub>c</sub> = 47.7

I have also calculated the value of  $M^2K$  and the quotient  $C$  of a number of other substances from the surface tension measurements of Ramsay and Shields. From the surface tension, the critical temperature may be calculated approximately by the rule of Eötvös, revised by Ramsay and Shields, namely,  $K(T_c - T - 6) = S(Mv)^{2/3}$ , where  $S$  is the surface tension in dynes at the absolute temperature  $T$  and  $Mv$  the molecular volume. Having thus found  $T_c$ , the fraction  $T/T_c$  may be calculated for any temperature and by interpolation from the curve expressing the relation between  $T/T_c$  and  $V/V_c$ ,  $V/V_c$  may be found and from this  $V_c$  may be calculated if  $V$  is known. We thus obtain the theoretical critical volume. From  $V_c$  and  $T_c$  the value of  $M^2K$  may be calculated either by the surface tension formula or the formula  $M^2K = V_c T_c 3.594 \times 10^{-40}$ . I shall, in the first place, show how exactly the law correlating  $M^2K$  with weight and valence holds for some substances which do not contain chlorine but contain elements of which the valence is fairly certain. Some of these substances associate slightly. The results are summarized in Table VII.

From Table VI it may be seen that the values obtained for  $C$ , although less reliable than those from the other tables, nevertheless agree well with the mean of about  $3 \times 10^{-37}$ .

The substances marked associating in the above table were found to be so by Dutoit and Mojoïu,<sup>1</sup> who determined the association and calculated the mean molecular weight at various temperatures.

There are two sources of uncertainty in computing " $a$ " and  $M^2K$  for the simplest gases; the first is the uncertainty of the critical data of some of them; and the second, the uncertainty that the coefficients of the formulas hitherto used for calculating these values remain the same for the gases. If the usual formula for " $a$ " be used, by which " $a$ " is calculated from the pressure and temperature, the formula being derived from certain assumptions as to the value of the crit-

<sup>1</sup> Dutoit and Mojoïu: Constante de capillarité et poids moléculaire. Jour. Chim. Phys., 7, 169 (1909).



TABLE VI

	Substance	Formula	Calc. $T_c$ Abs.	Calc. $V_c$	$\log_{10} M^*K$	Mol. wt.	No. of valences	$C \times 10^3$	Remarks
1	Pyridine	$C_5H_5N$	617.2	240	-35.72618	79	28	3.14	Associates
2	Aniline	$C_6H_7N$	677.9	279.6	-35.83315	93	34	3.16	"
3	Nitrobenzene	$C_6H_5NO_2$	715.4	323.5	-35.92012	123	36	3.08	"
4	Benzonitrile	$C_6H_5CN$	671	313	-35.87843	103	36	3.16	"
5	Quinoline	$C_8H_7N$	739.1	370	-35.99255	129	46	3.00	Normal
6	Guaicol	$C_8H_8O_2$	699.8	342	-35.93470	124	40	2.96	"
7	Paraldehyde	$C_3H_2O_3$	583.5	394	-35.91365	132	54	2.84	
8	Acetic anhydride	$C_4H_6O_3$	608.9	283	-35.79183	102	28	3.08	
9	Piperidine	$C_4H_{11}N$	604.4	292	-35.80236	85	34	3.24	Normal
10	Piperidine	$C_5H_{11}N$	604.4	$P_c = 45.9$	-35.79995	85	34	3.11	Guye and Mallet
11	Me propyl ketone	$C_6H_{10}O$	569.6	301	-35.79142	86	32	3.15	Associates
	Ethyl acetate	$C_4H_{10}O_2$	668	389	-35.96831	130	40	3.10	

ical coefficient, the value of  $C$  is, for fairly complex substances, about  $2.80 \times 10^{-37}$ ; but in the simple gases, such as  $N_2O$ ,  $H_2$ ,  $O_2$ ,  $N_2$ , etc., it is uniformly lower and about 2.25. The question is, therefore, whether this formula does not give an exact value for  $M^2K$ , or whether the relation of  $M^2K$  to the weight and valence is only approximate and does not remain the same for the simple substances. It is possible that the ratio 27/64 in the "a" formula is not the same for all substances, but may vary a little with the complexity, or compressibility, of the molecule. To solve this uncertainty, I have computed  $M^2K$  directly from the surface tension of the liquid gases by the half empirical formula which holds pretty well for more complex substances:  $M^2K(1/v_1 - 1/v_v) = 3Sv_1^{2/3}[(T_c - 6)/(T_c - T - 6)]^{2/3}$ . I have used the data of Baly and Donnan in this calculation. I also calculated  $M^2K$  from the latent heat of vaporization from figures given by Dewar in his article on Liquid Gases in the Encyclopedia Britannica, by the formula:  $L - E = a(1/V_1 - 1/V_v)$ .  $a = N^2M^2K$ . This formula neglects the heat used in the expansion of the molecules in passing from the liquid to the vapor. It generally gives, therefore, a value of  $M^2K$  somewhat too high. I have also computed  $M^2K$  by the usual formulas involving  $V_c$  and  $T_c$  instead of  $P_c$  such as the surface tension formula. In Table VII I have included the values thus calculated for hydrogen, using successively the critical data of Olszewski and Dewar.

It is evident that a considerable uncertainty in the case of hydrogen arises from the uncertainty of the critical data. I think the  $P_cV_c^2$  formula gives too high results. If we take the mean of all it would be  $-37.88161$ . With the valence 2 and the weight 2 this would give for "c" a value of  $3.02 \times 10^{-37}$ , which is close to the value obtained before.

I have made similar calculations in the case of oxygen, nitrogen, carbon dioxide and nitrous oxide. For the sake of brevity I omit these and give in Table VIII only the mean value for  $M^2K$  for each gas.

It is clear from Table VIII that with the exception of car-



TABLE VII  
Values of  $M^2K$  for hydrogen

$\text{Log}_{10} M^2K$	Formula	Critical data used	
—37.82276	Latent heat	$T_c = 32;$	Dewar
—37.95621	$M^2K = V_c^2 P_c 1.6296 \times 10^{-47}$	$P_c = 15; V_c = 60.42$	
—37.80014	$M^2K = 9.045 \times 10^{-16} (T_c - 6)^{20}$	$T_c = 32; P_c = 15; V_c = 60.42$	
—37.84190	$M^2K = V_c T_c^3 \cdot 594 \times 10^{-40}$	$T_c = 32; P_c = 15; V_c = 60.42$	
—37.87758	$M^2K = V_c T_c^3 \cdot 594 \times 10^{-40}$	$T_c = 38.5; P_c = 20; V_c = 54.52$	
—37.99118	$M^2K = P_c V_c^2 1.6296 \times 10^{-47}$	$T_c = 38.5; P_c = 20; V_c = 54.52$	Olszewski
—37.85242	$M^2K = 9.045 \times 10^{-16} (T_c - 6)^{20}$	$T_c = 38.5; P_c = 20; V_c = 54.52$	

TABLE VIII

Substance	Formula	Log <sub>10</sub> M <sup>2</sup> K	Mol. wt.	No. of valen-ces	C × 10 <sup>37</sup>	Formula used
1 Hydrochloric acid	HCl	-36.88550	36.45	2	4.49	Associates
2 Hydrogen selenide	H <sub>2</sub> Se	-35.14572	81.2	4	2.96	V <sub>c</sub> = 94.9; T <sub>c</sub> = 138; P <sub>c</sub> = 91
3 Sulphurous anhydride	SO <sub>2</sub>	-35.27880	64	8	2.97	T <sub>c</sub> = 156; P <sub>c</sub> = 78.9; V <sub>c</sub> = 123.1
4 Nitrous oxide	N <sub>2</sub> O	-35.09684	44	6(?)	3.04	P <sub>c</sub> = 75; d <sub>c</sub> = 0.41; T <sub>c</sub> = 35.4
5 Carbon dioxide	CO <sub>2</sub>	-35.08430	44	6	2.95	Mean value of M <sup>2</sup> K
6 Oxygen	O <sub>2</sub>	-36.65703	32	2(?)	2.84	"
7 Nitrogen	N <sub>2</sub>	-36.63671	28	2	2.96	"
8 Hydrogen	H <sub>2</sub>	-37.88161	2	2	3.02	"
9 Bromine	Br <sub>2</sub>	-35.14659	159.92	2	3.00	T <sub>c</sub> = 302.2; d <sub>c</sub> = 1.18
10 Chlorine	Cl <sub>2</sub>	-35.22351	70.9	6	2.96	T <sub>c</sub> = 146; P <sub>c</sub> = 93.5; V <sub>c</sub> = 111; Cl = 3
11 Carbon monoxide	CO	-36.65853	28	4	1.96	T <sub>c</sub> = -139.5; P <sub>c</sub> = 35.5; d <sub>c</sub> = 1.14
Carbon monoxide	CO	-36.65853	28	2(?)	3.11	



bon monoxide, which is hopelessly aberrant if carbon and oxygen have a higher valence than one, the value of C comes close to the value found in other substances, namely,  $2.98 \times 10^{-37}$ . It is true that in making the calculation I have, in one or two instances, used other valence numbers than those generally attributed to the elements. Thus chlorine is trivalent. But I shall show in a subsequent paper that chlorine is always trivalent in its organic compounds; nitrogen in the gaseous form is univalent, while it is bivalent in nitrous oxide;<sup>1</sup> but both these valences have already been ascribed to nitrogen. Oxygen cannot have more than two valences in the molecule. The value I have taken for  $M^2K$  is certainly as high as the facts warrant and the critical data seem reliable. I believe that the conclusion is justified that oxygen is monovalent in its gaseous form. The surprising fact is the value for carbon dioxide. The carbon cannot be more than bivalent here, giving a kind of peroxide formula, if carbon dioxide is to follow the rule. On the whole, the gases agree well with the results already obtained for C.

In a subsequent paper I shall show that all chlorine compounds also follow the rule if the chlorine be taken trivalent; and that there is good reason, from quite independent sources, to attribute a valence of three to chlorine. The valences of sulphur, nitrogen and the argon group will also be considered separately.

In closing it may be pointed out that the law just established can be used to determine the number of valences in a molecule if  $M^2K$  of the substance is known, or if its critical data are known, as follows: If the computation is made from the approximate values of "a" given in the Landolt-Börnstein-Meyerhoffer tables, which were computed by the formula:  $a = 27T_c^2/(64 \times 273^2 \times P_c)$ , proceed by the following formula: (1) Valence number =  $(a)^{3/2} \times 3.2 \times 10^5/(\text{Mol. Wt.})$ . Or, if the calculation is made from the critical data, the following formula will serve:

<sup>1</sup> Possibly nitrogen is univalent in this gas, but the oxygen is quadrivalent, having two free valences.

(2) Val. number =  $0.0043 T_c^3 / P_c^{3/2}$  (Mol. Wt.); or, if the calculation is made from the temperature and volume:

(3) Val. number =  $4.21 \times 10^{-5} \times (V_c T_c)^{3/2} / (\text{Mol. Wt.})$ .

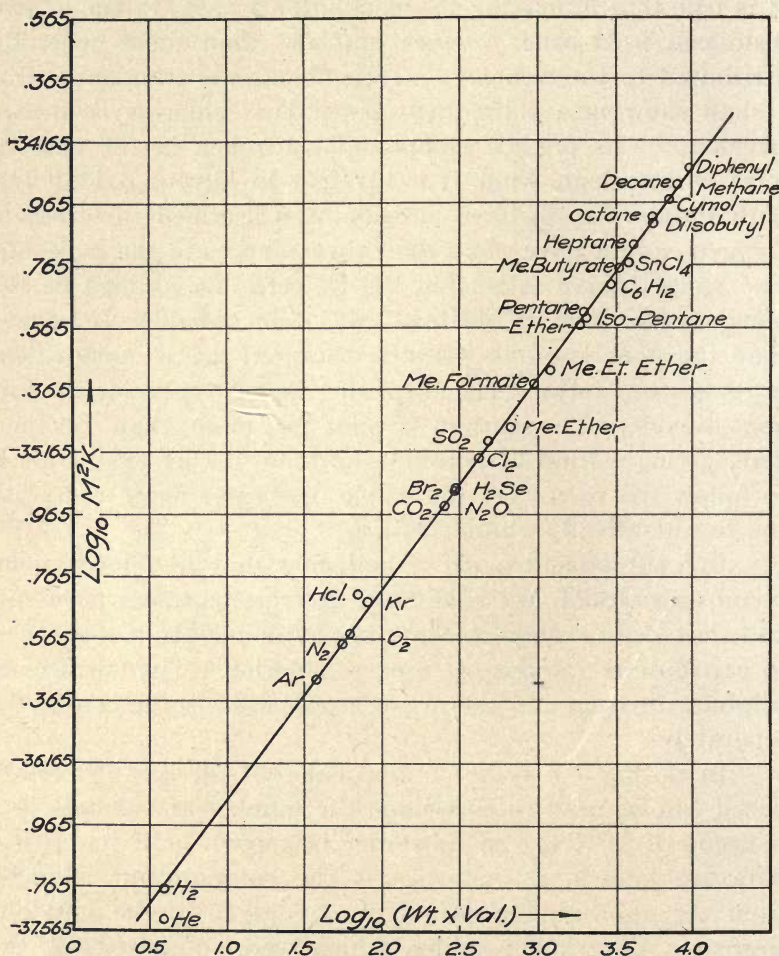


Fig. 1—Showing the relationship between the logarithm of  $M^2/K$  and the logarithm of the product of the molecular weight by the number of valences

In these formulas  $V_c$  is the critical volume of a gram mol. in cubic centimeters;  $T_c$ , the absolute critical temperature; and  $P_c$  the critical pressure in atmospheres.



How accurately the number of valences can be calculated by the first formula is shown by Table IX, which is, of course, little more than a rearrangement of the results already stated in the preceding tables, except that in this instance I have used the approximate values of "a" given in the Landolt tables computed by the formula  $a = 27T_c^2/(64 \times 273^2 \times P_c)$ .

An inspection of Table IX will, I think, convince all that molecular cohesion, as expressed by the value "a" of van der Waals' equation, is a function of the weight and valence of the molecule. If "a" is calculated for all these substances from the critical temperature and pressure by the formula:  $a = 27T_c^2/64P_c \times 273^2$ , the number of valences is calculated, with a remarkable degree of approximation, from the assumption that  $a = CN^2Wt^{2/3}Val^{2/3}$ , by the formula given at the beginning of the table. It will be noticed that all associating substances give by this formula a larger number of valences than that calculated for the normal substance, and that the degree of excess of the number of valences is more or less proportional to the degree of association. Thus the largest excess is in the case of water, where the cohesion calls for 22 valences, and in some of the nitriles; whereas substances like the esters, which associate very little, have only a few valences in excess. Putting aside associating substances, of which the deviation from the rule is to be expected, there are certain exceptions characteristic of certain observers. The coefficient  $3.2 \times 10^5$  was taken from Young's data for ether. It will be seen that always Nadejdine's critical constants give a value for "a" lower than Young's, where both observers have worked on the same substances. So the substances computed from Nadejdine's critical data generally show a deficit of two or three valences to the molecule. I think in these cases Young's values are to be accepted. Vincent and Chappuis' determinations, also, generally come a little low, though they are very close. The main constant deviation from the law is to be observed in the case of substances like methane and hydrogen of very low molecular weight and great molecular simplicity; and those substances

TABLE IX

Computation of the number of valences per molecule from the molecular cohesion by the formula: Valences =  $a^{3/2} \times 3.2 \times 10^5 / (\text{molecular weight})$

Substance	Formula	Value of "a"	Computed valences	Theoretical valences	Observer	Remarks
Ether	$C_2H_{10}O$	0.03473	28	28	Young	
Ethyl acetate	$C_4H_8O_2$	0.04076	29.9	28	Young	
Ethyl acetate	$C_4H_8O_2$	0.03897	27.98	28	Nadejdine	
Ethane	$C_2H_6$	0.01060	11.64	14	Olszewski	
Ethane	$C_2H_6$	0.01189	13.83	14	Dewar	
Acetone	$C_3H_6O$	0.02774	25.49	20	Sajotschewsky	Associated
Acetone	$C_3H_6O$	0.02459	21.3	20	Sajotschewsky	
Acetylene	$C_2H_2$	0.008745	10.06	10		
Acetonitrile	$C_2H_3N$	0.03503	51.2	14	Guye & Mallet	Associated
Ethyl amine	$C_2H_7N$	0.01736	16.3	18	Vincent & C.	
Ethyl benzene	$C_8H_{10}$	0.05701	41.09	42	Altschul	
Ethyl butyrate	$C_8H_{12}O_2$	0.05993	40.47	40	Nadejdine	
Ethyl chloride	$C_2H_5Cl$	0.02174	15.92	16	Vincent & C.	Cl = 3
Ethylene	$C_2H_4$	0.00889	9.58	{ 12 (?) 10 (?) }	Dewar	One carbon bivalent
Ethylene	$C_2H_4$	0.00877	9.39	18	Olszewski	Cl = 3
Ethylene chloride	$C_2H_4Cl_2$	0.03370	20.02	22		Associated
Ethyl formate	$C_3H_6O_2$	0.03122	23.85	22	Young	
Ethyl formate	$C_3H_6O_2$	0.02949	21.91	22	Nadejdine	
Ethylidene chloride	$C_2H_4Cl_2$	0.03090	17.57	18	Nadejdine	Cl = 3
Ethyl isobutyrate	$C_6H_{12}O_2$	0.05754	38.1	40	Nadejdine	
Ethyl propionate	$C_5H_{10}O_2$	0.05088	36.0	34	Young	Associated
Ethyl propionate	$C_5H_{10}O_2$	0.04861	33.62	34	Nadejdine	
Alcohol	$C_2H_6O$	0.02395	25.8	16	Young	Associated



TABLE IX (Continued)

TABLE IX (Continued)

Substance	Formula	Value of "q"	Computed valences	Theoretical valences	Observer	Remarks
Isobutyl alcohol	$C_4H_{10}O$	0.03394	27.04	28	Nadejdine	
Isobutyl benzene	$C_{10}H_{14}$	0.07692	50.95	54	Altschul	
Isobutyl formate	$C_5H_{10}O_5$	0.04492	29.9	34	Nadejdine	
Isopentane	$C_5H_{12}$	0.03650	31.0	32	Young	
Isopropyl alcohol	$C_3H_8O$	0.02747	24.3	22	Nadejdine	
Isopropyl benzene	$C_9H_{12}$	0.07105	50.5	48	Altschul	
Iodobenzene	$C_6H_5I$	0.06590	42.1	42	Young	
Carbon dioxide	$CO_2$	0.00719	4.43	6?	Dewar	
Carbon monoxide	$CO$	0.00285	1.28	2?	Olszewski	
Carbonoxysulfide	$COS$	0.00784	3.70	6?	Olszewski	
Mesitylen	$C_9H_{12}$	0.06840	47.7	48	Altschul	
Methane	$CH_4$	0.00376	4.6	8	Olszewski	
Methyl acetate	$C_3H_6O_2$	0.03206	24.8	22	Young	Slt. association
Methyl acetate	$C_3H_6O_2$	0.03047	23.0	22	Nadejdine	
Methyl ether	$C_3H_8O$	0.01609	14.2	16	Leduc & S.	
Methyl ethyl ether	$C_3H_8O$	0.02381	19.6	22	Nadejdine	
Methyl alcohol	$CH_3O$	0.01895	26	10	Young	Association
Methyl amine	$CH_5N$	0.01441	17.9	12	Vincent & C.	Association
Methyl butyrate	$C_5H_{10}O_2$	0.05082	35.9	34	Young	
Methyl chloride	$CH_3Cl$	0.01332	9.8	10	Vincent & C.	Cl = 3 Fl = 1
Methyl fluoride	$CH_3Fl$	0.00923	8.4	8	Collie	Association
Methyl formate	$C_2H_4O_2$	0.02371	19.47	16	Young	
Methyl formate	$C_2H_4O_2$	0.02160	16.9	16	Nadejdine	
Methyl isobutyrate	$C_5H_{10}O_2$	0.04882	33.8	34	Young	
Methyl propionate	$C_4H_8O_2$	0.03968	28.75	28	Young	
Methyl valerate	$C_6H_{12}O_2$	0.05771	38.2	40	Nadejdine	
Naphthalin	$C_{10}H_8$	0.07928	55.8	48	Guye & Mallet	
Octane	$C_8H_{18}$	0.07284	55.2	50	Young	



TABLE IX (Continued)

		0.03789	32.8	32	Young Guye & Mallet Leduc & S.	Some association Association
Pentane	$C_5H_{12}$	0.03789	32.8	32	Young	
Phenetole	$C_8H_{10}O$	0.07009	48.7	44	Guye & Mallet	
Phosphorus hydride	$PH_3$	0.00939	8.6	6	Leduc & S.	
Propane	$C_3H_8$	0.01760	17.0	20	Olszewski	
Propionitrile	$C_3H_5N$	0.04279	51.5	20	Guye & Mallet	Association
Propyl acetate	$C_5H_{10}O_2$	0.05149	36.7	34	Young	Slt. association
Propyl acetate	$C_5H_{10}O_2$	0.04908	34.1	34	Nadejdine	
Propyl alcohol	$C_3H_8O$	0.03250	31.25	22	Young	Associated
Propyl amine	$C_3H_9N$	0.02729	24.45	24	Vincent & C.	
Propyl benzene	$C_9H_{12}$	0.07146	50.9	48	Altschul	
Propyl chloride	$C_3H_7Cl$	0.02819	19.31	20	Vincent & C.	Cl = 1
Propyl formate	$C_4H_8O_2$	0.04086	29.7	28	Young	Slt. association
Pseudo-cumole	$C_9H_{12}$	0.07298	52.6	48	Altschul	
Oxygen	$O_2$	0.00273	1.4	2?	von Wroblewski	
Carbon bisulphide	$CS_2$	0.02316	14.8	16	Batelli	S = 6
Hydrogen sulphide	$H_2S$	0.00876	7.72	4	Leduc & S.	Associated
Sulphur dioxide	$SO_2$	0.01316	7.6	8	Sajotschewsky	S = 4
Hydrogen selenide	$H_2Se$	0.01050	4.2	4	Olszewski	N = 1; O = 1
Nitric oxide	$NO$	0.00257	1.4	2(?)	Olszewski	N = 2; or N = 1
Nitrous oxide	$N_2O$	0.00710	4.35	4(?)	Villard	
				2(?)		
Nitrogen	$N_2$	0.00277	1.67	2(?)	Olszewski	N = 1
Thiophen	$C_4H_4S$	0.04130	32	22	Pawlewski	Associated
Toluene	$C_7H_8$	0.04795	36.5	36	Altschul	
Triethylamine	$C_6H_{15}N$	0.05415	39.9	42	Vincent & C.	
Trimethylamine	$C_3H_7N$	0.02594	22.7	24	Vincent & C.	
Water	$H_2O$	0.01149	21.9	4	Cailletet & C.	Associated
Hydrogen	$H_2$	0.00042	1.38	2	Olszewski	
o-Xylol	$C_8H_{10}$	0.05974	44.1	42	Altschul	
Stannic chloride	$SnCl_4$	0.05365	15.25	16	Young	Cl = 3

studied by Guye and Mallet<sup>1</sup> of very high molecular weight and complexity, such as durol, cymol, diphenyl and diphenyl methane. So far as I can find, the evidence is that these substances associate but little; so the deviation cannot be attributed to that. It may be that Guye and Mallet's determinations have some constant source of error which brings them higher than determinations on similar substances by Altschul, but I do not think this is the explanation of the facts. The fact that the deviation is in the opposite direction in the simplest from what it is in the most complex substances leads me to believe, as stated on page 192, that the calculation of "*a*" by this formula may be at fault.

While there can be little doubt that this formula gives a value for "*a*" approximately correct for all normal substances, as van der Waals has recently reaffirmed, and accordingly that the ratio 27/64 is at least approximately true for all substances, yet it is not certain that this ratio is exactly constant for all substances. It can only be strictly justified for the simplest substances in which "*b*," the volume of the molecule, is constant. I believe it to be far more likely that this ratio changes a little with progressive increase in molecular complexity, and a resulting greater compressibility of the molecule, as indeed van der Waals has suggested, than that the law, which I have here attempted to establish, of the dependence of cohesion on molecular weight and valence holds strictly only for substances of medium complexity. This relationship of cohesion to these two molecular properties seems so probable and so fundamental that I believe we may, with some confidence, anticipate that if it holds at all, it holds everywhere.

It seems not worth while to consider this question further until it is decided whether "*a*" is in all instances determined by the formula just mentioned; or whether the ratio 27/64 is true only for the simplest substances with constant molecular volume, that is a constant "*b*;" and that for more complex

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<sup>1</sup> Guye and Mallet: *Comptes rendus*, 133, 1288 (1901); 134, 168 (1902).



and compressible molecules it must be slightly diminished as the complexity and compressibility increases. If the latter shall prove to be the case, then most of the exceptions noted in the preceding pages would disappear. The substitution of the value for " $M^2K$ " computed by my formula from the surface tension would have given a much closer agreement of the calculated and observed valence numbers, but I wished to show that the law holds at least approximately even though we use the approximate values of " $a$ " computed by the usual formula.

The constant  $2.98 \times 10^{-37}$  discovered in the foregoing pages, is evidently the factor  $M^2K$  of a substance of unit molecular weight and unit valence. No such substance as this is known, but hydrogen with a weight and valence of two, and helium with a weight of four and valence of unity(?) have values of  $M^2K$  not very different. Thus for hydrogen,<sup>1</sup>  $M^2K$  is between  $3.16$  and  $7.72 \times 10^{-37}$ ; and  $M^2K$  of helium lies between the same two values probably. The value of  $2.98 \times 10^{-37}$  is of the order of magnitude of the gravitational attraction of two average molecules. Thus at  $20^\circ$  two molecules of ether in the liquid state attract each other gravitationally with a force of  $3.11 \times 10^{-37}$  dynes. The similarity of these values is, however, probably only a coincidence.

### Conclusion

The facts presented in the foregoing pages enable us to draw the general conclusion: The "mass" of cohesion of a molecule is everywhere proportional to the cube root of the molecular weight multiplied by the cube root of the number of valences in the molecule. Or, to put it in another way, " $a$ " of van der Waals' equation for one cc. of gas under standard conditions is equal to  $2.98 \times 10^{-37} \times \text{Mol. Wt}^{2/3} \times \text{Valences}^{2/3} \times (2.77 \times 10^{19})^2$  dynes; or this number divided

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<sup>1</sup>  $M^2K$  for  $H_2$  computed from the value " $a$ " given by Landolt-Börnstein is  $5.55 \times 10^{-37}$ . This was computed by the formula  $a = \frac{27}{64} \frac{T_c^2}{273^2 \times P_c}$ .

by  $1.0135 \times 10^6$  atmospheres. This formula gives a value for "a" somewhat higher than the ordinary formula and it may be that the coefficient should be taken a little lower.

The theoretical significance of this relationship of cohesion to the molecular weight and the number of valences is very interesting, but I shall reserve its consideration for a subsequent paper.

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# THE VALENCE OF CHLORINE AS DETERMINED FROM THE MOLECULAR COHESION OF CHLORINE COMPOUNDS

BY ALBERT P. MATHEWS

Since molecular cohesion is a function of the molecular weight and the number of valences in the molecule,<sup>1</sup> we may use the cohesion for the purpose of determining the valence of elements; and in this paper I shall consider chlorine, although something will be said, also, about the other halogens.

It is generally believed, at the present time, that the valence of chlorine is not fixed, but varies in different compounds from one to seven. In its organic, and some inorganic compounds, and in its elemental form it is generally represented as univalent; whereas in the chlorates it is supposed to be pentavalent; and in the perchlorates it is heptavalent.

That chlorine even in such compounds as chloroform, where it replaces univalent hydrogen, may not be univalent is indicated by the action of chlorine compounds on light. Drude,<sup>2</sup> reasoning that it must be the valence electrons of compounds which would have a period of vibration sufficiently long to respond to light waves, worked out a modification of the Ketteler-Helmholtz dispersion formula which enabled an approximate computation of the number of electrons influencing dispersion in the molecule. He found that in many cases this number was close to the total number of valences in the molecule; but in the case of compounds containing chlorine and fluorine, the number of such light-refracting valences was always greater than in the corresponding hydrogen compounds, and he inferred from this that

<sup>1</sup> Mathews: "The Relation of the Constant "*a*" of van der Waals' Equation to the Molecular Weight and the Number of Valences in the Molecule," *Jour. Phys. Chem.*, **17**, 181 (1913).

<sup>2</sup> Drude: "Optische Eigenschaften und Elektronen Theorie," *Annalen der Physik*, [4], **14**, 677 (1904).



these elements must be polyvalent, and not monovalent, as they were usually supposed to be. This conclusion of Drude's was confirmed by Pascal<sup>1</sup> both by the dispersion method of computing valence and by a study of the diamagnetic properties of halogen compounds, the diamagnetic properties having been shown to be related to the number of valences in the molecule. Pascal concluded that fluorine, in organic compounds at any rate, was univalent; but chlorine and the other halogens were polyvalent, and probably chlorine was trivalent. Traube,<sup>2</sup> in a study of the relationship between the molecular refraction of compounds and the number of their valences, found that for most compounds the molecular refraction of Brühl divided by the number of valences in the molecule was a constant, or nearly such, in all saturated compounds; but in the case of molecules containing the halogens it was necessary to ascribe several valences to the halogens to obtain this constant. He attributed seven valences to chlorine, and had to make still other assumptions for bromine and iodine to bring them into line.

Several chemists, also, have in the past ascribed several valences to chlorine. Thus Meldola<sup>3</sup> wrote the formula of

methylether hydrochloride, in the form  $\begin{array}{c} \text{CH}_3 \\ \diagup \\ \text{CH}_3 \end{array} \text{O} = \text{Cl}-\text{H}$ ,

with chlorine trivalent; Nef<sup>4</sup> represented elemental chlorine as trivalent, but combined chlorine generally as monovalent; and recently Thiele<sup>5</sup> has especially emphasized the reserve, or extra, valences of iodine and bromine, although, as a rule, he represents chlorine as univalent. Even in sodium chloride it is not certain that the chlorine is univalent, since it is

<sup>1</sup> Pascal: "Recherches magnéto-chimiques sur la structure atomique des halogenes," *Comptes rendus*, **152**, 862 (1911); "Sur un mode de controle optique des analyses magnéto-chimiques," *Ibid.*, **152**, 1852 (1911).

<sup>2</sup> Traube: "Valency, Lichtbrechung u. volume," *Ber. chem. Ges. Berlin*, **40**, 130 (1907).

<sup>3</sup> Meldola: I have mislaid this reference and have not been able to find it again.

<sup>4</sup> Nef: *Liebig's Ann.*, **298**, 205 (1897).

<sup>5</sup> Thiele and Peter: *Ber. chem. Ges. Berlin*, **38**, 2842 (1905).

known that sodium chloride will add iodine, presumably by the extra valences of the chlorine.<sup>1</sup>

There is good ground, therefore, for doubting whether chlorine is ever monovalent. This question can be tested easily by the cohesion method.

Before proceeding to the actual computations it must be decided whether the cohesion method detects only valences actually employed in binding atoms together, or stretching between the atoms; or whether it detects in addition the reserve valences; and also valences which do not extend to atoms, but which are open, in an active form, and ready to combine if the opportunity arises. It is clear from my former paper that concealed, polarized, resting or reserved valences do not play any part in cohesion; or, at any rate, they are not to be counted in the number of valences affecting the cohesion. Thus oxygen has certainly two reserve valences which are usually in an inactive or resting state. In many compounds examined, not more than two valences could be attributed to the oxygen as affecting its cohesion. These two reserve valences played no rôle as long as they were inactive. Similarly, nitrogen has the power of opening up at least seven valences, but it was actually found that only one, two or three valences played a rôle in the cohesion of the nitrogen compounds, depending on how many active valences the atom had. The reserve, or inactive, valences played no part. Carbon is usually quadrivalent, but it is suspected of having the power of becoming hexavalent; but the number of valences active in carbon compounds was always two, or four. If these reserve valences of carbon exist they do not affect cohesion. Sulphur, too, although it may be hexavalent, has only four of its valences playing a part in the cohesion of sulphur dioxide; the two reserve valences are inactive on the cohesion.

It is clear, then, that the cohesion does not detect, and consequently it is not affected by, those reserve valences which are polarized, or resting, or, which are, as it were, like antennae, withdrawn or folded, within the atom.

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<sup>1</sup> See Friend: "The Theory of Valency," London, 1909, pp. 58 et seq.



But valences may conceivably exist in an active state, not stretching between atoms, but extending outward from the atom and in a condition to unite with other atoms. These are active valences. For example, we may expect the valences on the atoms of a dissociated, monovalent gas to be in this condition. Such atoms would naturally be very active chemically and we should expect the cohesion of such particles to be affected by this condition. There are many evidences that this is actually the case and that valences of this kind are detected by cohesion and will be included in the number of valences computed from the cohesion as existing in the molecule. This is well shown in the argon group, which I shall discuss later, in which it appears that there are two such active valences in argon, krypton, and probably xenon. It appears to be the case, too, in unoxidized sulphur compounds such as ethyl sulphide, as I shall show in a subsequent paper. And there is evidence elsewhere that these open or active valences affect cohesion, although they do not stretch between the atoms of the same molecule. It is, then, active valences, and valences actually employed in binding together the atoms of the molecule, which affect molecular cohesion. It is only the number of such valences which the cohesion enables us to compute, and it is, of course, exactly for this reason that the method has so great a value.

We may then be certain that if we find the valence of chlorine to be three and the compounds are not associating, those three valences are not free, but are actually extending between atoms in the molecule, and if we wish an accurate graphic formula of the compound we must represent these ties.

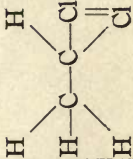
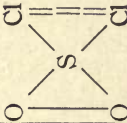
The method of measuring the number of valences is to compute the number from "*a*" of van der Waals' equation, or from what I have called the square of the cohesive mass, or  $M^2K$ , a factor which is equal to "*a*", divided by the square of the number of molecules in the volume of fluid for which "*a*" has been taken. The method of computing  $M^2K$  is given in the previous paper. The formula employed is  $M^2K = 2.98 \times 10^{-37} (\text{Mol. Wt.} \times \text{Valences})^{2/3}$ . Or: Number of Valences =  $(M^2K)^{3/2} \times 6.147 \times 10^{54} / (\text{Mol. Wt.})$ .

TABLE I.—THEORETICAL AND COMPUTED NUMBER OF VALENCES IN CHLORINE COMPOUNDS

1 Substance	2 Formula	3 $\text{Log}_{10} M^{\circ}K$	4 Mol. wt.	5 Computed No. of valences	Theoretical No. of valences		8 Graphic formula
					6 $\text{Cl} = 3$	7 $\text{Cl} = 1$	
1 Carbon tetrachloride	$\text{CCl}_4$	—35.74247	153.8	16.4	16	8	
2 Stannic chloride	$\text{SnCl}_4$	—35.87500	260.8	15.3	16	8	
3 Germanium tetrachloride	$\text{GeCl}_4$	—35.79817	214.3	14.3	16	8	
4 Chlorobenzene	$\text{C}_6\text{H}_5\text{Cl}$	—35.84615	112.45	32.1	32	30	
5 Chloroform	$\text{CHCl}_3$	—35.61128	119.35	13.5	14	8	
6 Methyl chloride	$\text{CH}_3\text{Cl}$	—35.26811	50.45	9.7	10	8	



TABLE I (Continued)

7	Propyl chloride	$C_3H_7Cl$	-35.59465	78.45	19.7	22	20	
8	Ethyl chloride	$C_2H_5Cl$	-35.49356	64.45	16.6	16	14	
9	Ethylidene chloride	$C_2H_4Cl_2$	-35.63775	98.9	17.8	18	14	
10	Ethylene chloride	$C_2H_4Cl_2$	-35.67488	98.9	20.2	18	14	
11	Chlorine	$Cl_2$	-35.22351	70.9	5.9	6	2	$Cl \equiv Cl$
12	*Silicon tetrachloride	$SiCl_4$	-35.74648	170.2	15.1	16	8	
13	*Thiosulphuryl chloride	$S_2Cl_2$	-35.76597	134.9	20.3	18	14	Some association. S=6
14	*Acetyl chloride	$CH_3COCl$	-35.55497	78.45	16.8	16	14	
15	*Chlorethyl formate	$C_2H_5ClO_2$	-35.74968	108.45	23.9	24	22	
16	*Chloral	$CCl_3CHO$	-35.73899	147.35	16.9	20	14	
17	*Thionyl chloride	$SOCl_2$	-35.63703	118.9	14.8	14	10	
18	*Sulphuryl chloride	$SO_2Cl_2$	-35.65648	134.9	14.1	14	10	
19	*Phosphorus trichloride	$PCl_3$	-35.70915	137.35	16.4	16	10	P = 7
20	*Phosphorus oxychloride	$POCl_3$	-35.76967	153.35	18.1	18	12	P = 7
21	Hydrochloric acid	HCl	-35.88550	36.45	3.6	4	2	Associates
22	Bromine	$Br_2$	-35.14659	159.92	2.0	6	2	Br-Br
23	Brombenzene	$C_6H_5Br$	-35.89265	157	27.0	32	30	
24	Iodobenzene	$C_6H_5I$	-35.96071	204	26.4	32	30	
25	*Ethyl iodide	$C_2H_5I$	-35.67614	155.85	12.9	16	14	

\*Compounds marked \* have  $V_c$  and  $T_c$  computed from the surface tension, and  $M^2K$  is not so accurate as when  $V_c$  and  $T_c$  are directly determined. For the method of computation see the preceding paper.

To show how closely these compounds yield the constant "*c*," where  $c = M^2K/(\text{Mol. Wt.} \times \text{Valences})^{2/3}$ , Table II is appended. "*C*" was found in a previous paper to be about  $2.98 \times 10^{-37}$  for other than chlorine compounds. Chlorine is throughout considered as trivalent.

TABLE II.—"*C*" CALCULATED FOR CHLORINE COMPOUNDS. CL CONSIDERED TRIVALENT EXCEPT IN HYDROCHLORIC ACID AND PROPYL CHLORIDE

Substance	Formula	Valance	$C \times 10^{37}$	Remarks
1 Carbon tetrachloride	$\text{CCl}_4$	16	3.03	
2 Stannic tetrachloride	$\text{SnCl}_4$	16	2.91	
3 Germanium tetrachloride	$\text{GeCl}_4$	16	2.78	All 4 chlorines trivalent
4 Silicon tetrachloride	$\text{SiCl}_4$	16	2.86	
5 Chloroform	$\text{CHCl}_3$	14	2.90	
6 Methyl chloride	$\text{CH}_3\text{Cl}$	10	2.97	
7 Chlorine	$\text{Cl}_2$	6	2.96	
8 Ethyl chloride	$\text{C}_2\text{H}_5\text{Cl}$	16	3.06	
9 Ethylene chloride	$\text{C}_2\text{H}_4\text{Cl}_2$	18	3.15	
10 Ethylidene chloride	$\text{C}_2\text{H}_4\text{Cl}_2$	18	3.02	
11 Chlorobenzene	$\text{C}_6\text{H}_5\text{Cl}$	32	2.98	
12 Thiosulfuryl chloride	$\text{S}_2\text{Cl}_2$	18	3.23	Some association
13 Acetyl chloride	$\text{CH}_3\text{COCl}$	16	3.08	
14 Chlorethyl formate	$\text{C}_3\text{H}_5\text{ClO}_2$	24	2.96	Slight association
15 Chloral	$\text{CCl}_3\text{CHO}$	20	2.67	
16 Thionyl chloride	$\text{SOCl}_2$	14	3.09	Slight association (sulphur hexavalent)
17 Sulfuryl chloride	$\text{SO}_2\text{Cl}_2$	14	2.97	
18 Propyl chloride	$\text{C}_3\text{H}_7\text{Cl}$	21	2.93	Sulphur quadrivalent
19 Phosphorus trichloride	$\text{PCl}_3$	16	3.03	
20 Phosphorus oxychloride	$\text{POCl}_3$	18	2.99	
21 Hydrochloric acid	$\text{HCl}$	2	3.24	Association (?)

Mean (omitting  $\text{HCl}$ ,  $\text{S}_2\text{Cl}_2$  and  $\text{CCl}_3\text{CHO}$ ), 2.99

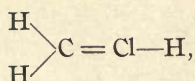
The answer to the question whether chlorine is trivalent or monovalent is given in no indecisive manner by the method



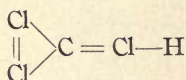
of determining the valence from the cohesion, as is shown in columns 5 and 6 of Table I. With three possible exceptions, chlorine is seen to be everywhere trivalent. The possible exceptions are hydrochloric acid, propyl chloride and one of the chlorine atoms in germanium tetrachloride. In the first, association renders the valence of the chlorine somewhat doubtful; in other words, here also chlorine may be trivalent. In the second, propyl chloride, the critical data may be wrong. They were determined in 1886 by Vincent and Chappuis, and the other determinations by these authors give generally a value for "*a*" slightly lower than is to be expected. It is not impossible, therefore, that a redetermination of the critical data for this substance will make  $M^2K$  sufficiently high to make the chlorine trivalent. For the third substance, germanium tetrachloride, I can find but one determination of the critical data in 1887. It is not unlikely, therefore, that the data will need some revision. This method of determining the valence of chlorine confirms, therefore, the conclusions of Pascal, based on the study of the dispersion and the magnetic properties, that chlorine is polyvalent, and, further, this method shows it to be beyond doubt generally trivalent.

Moreover, since nearly all these compounds are normal and not associating, the valences of the chlorine are shown to be not resting, or in reserve, and not dissociated active valences, but actually extending between the atoms of the molecule. I have indicated in column 8 of Table I, some possible structural formulae showing how these valences may be extending in the molecule. Where there are two or more chlorine atoms in the molecule, no serious reconstruction of the graphic formulae is required, since the extra valences may be pictured as reaching between the chlorine atoms; but where there is an odd number of chlorine atoms in the molecule, or where there is but a single one, then a fundamental change must occur in the graphic formula. For example, in ethyl and methyl chlorides, the formula must be written as I have indicated, with the chlorine joining the carbon by two bonds

and with one hydrogen united to the chlorine. I do not wish to lay stress on this point until by a careful determination of the critical data the number of valences in the molecule shall have been exactly determined, but it may be pointed out that, if methyl chloride be written as:



the reason why it decomposes into methylene and hydrochloric acid appears at a glance, since such a double bond is always a source of weakness; and similarly with ethyl chloride, which would be in reality ethylidene chlorhydrate, decomposing into ethylidene and hydrochloric acid. One can also more easily understand in this way the decomposition of chloroform into dichloro-methylene and hydrochloric acid, as the formula



shows. Phosgen will arise from the dichlormethylene uniting with oxygen.

The evidence, then, from such various sources as the behavior toward light, the diamagnetic properties and cohesion is unanimous that chlorine is polyvalent and not monovalent; many of the chemical and physiological properties of chlorine compounds are also more easily understood on the hypothesis of its trivalency. We may, therefore, conclude that in all these compounds chlorine is trivalent.

The question which must now be settled is no longer whether chlorine is trivalent, but whether it is ever monovalent. It is certainly trivalent in most of these compounds in which it was supposed to be monovalent; it is trivalent even in its elemental state. It remains to be seen whether it is ever monovalent. In hydrochloric acid it would appear to be monovalent; but it is exactly here that association takes place. Is it without significance that exactly that compound associates which has but one of the valences of the chlorine



satisfied by another monovalent atom? Is it not rather more probable that this is the cause of its association, the other two valences being not closed, but out and active? The computation actually shows that the chlorine is here also trivalent. I know of no means of telling whether it is monovalent or trivalent in sodium chloride. But it is not impossible that sodium chloride itself is a highly associated substance. Furthermore, its power of adding iodine indicates that the chlorine may be trivalent. Friend also states that sodium chloride may be  $\text{Na} - \text{Cl} = \text{Cl} - \text{Na}$ .

Concerning the valence of the other halogens, the facts are too scanty and the data too unreliable to draw a conclusion from the cohesion, except perhaps in the case of elemental bromine, which appears to be univalent. The critical data of brombenzene and iodobenzene were not directly determined by Young, but computed from the temperature, pressure and density curves. I do not believe that they are entirely trustworthy, since the number of valences found in the molecule is too small even if these halogens are considered monovalent, unless the carbon be here trivalent, and this does not seem possible.  $T_c$  and  $V_c$  of ethyl iodide, I computed from Ramsay and Shields' surface-tension determinations, and this computation is not very accurate. Hence I do not attach much weight to the cohesive evidence of the valence of any of these compounds of bromine and iodine. There are no indications, however, that they are polyvalent. That they are polyvalent is, however, indicated from their action on light, their diamagnetic properties and many of their chemical properties. Inasmuch, however, as the refraction method is not very satisfactory for determining valence, the question of the valence of these substances must be left open, with the probability that they will be found to be polyvalent like chlorine.<sup>1</sup>

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<sup>1</sup> The fact that bromine is monovalent in its elemental state may account for its relative inertness and is confirmed by its dissociation at high temperatures, when the atoms have been shown to have but one active valence. See Friend: "The Theory of Valence," 1909, p. 18.

Fluorine is apparently monovalent in fluorbenzene, since even with fluorine monovalent the number of valences computed from the cohesion is still too small. For this I can give no reason since the critical data of this substance seem to be accurately known. In methyl fluoride the total valences are computed as 9, whereas there should be 10 if fluorine is trivalent and 8 if it is monovalent. Pascal found fluorine to be monovalent by the magnetic and optical method; but Drude, from the optical behavior of calcium fluoride, believed it to be polyvalent. The critical data of more fluorine compounds must be accurately determined before the cohesional method can determine the valence of fluorine. The chemical behavior of hydrogen fluoride leaves no doubt that in it fluorine is polyvalent.

There is still another interesting conclusion from this study: it appears that all substances, and only those substances, associate, which are found by this method to contain active, free valences. I believe we may here have the explanation of the cause of association; and possibly the reason why associating substances dissolve in other associating liquids and are there normal, but as this is a separate problem in itself, I shall hope to return to it later.

### Summary and Conclusion

1. If the valence of chlorine be determined by the cohesional method it is found to be trivalent in its elemental state and in nearly all the compounds examined. The three valences of the chlorine in these compounds are not reserve valences, but are all in action and extending between the atoms of the molecule. Graphic formulae have been suggested based on this fact.

2. This result is in harmony with the determination of the valence of chlorine by the diamagnetic and refraction method.

3. The valence of fluorine is more doubtful, but appears to be unity in fluorbenzene. Bromine has unity valence in its elemental form. The valence of iodine and bromine in



their compounds cannot be definitely determined from their cohesion on account of the inadequacy of the critical data.

4. The cohesional method detects two kinds of valences, namely, valences actually extending between the atoms and active in binding the atoms together; and valences active or open, which are in a position to unite, but to which no atoms are attached. Reserved, or resting, valences play no part as valences in molecular cohesion.

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# THE VALENCE OF OXYGEN, SULPHUR, NITROGEN AND PHOSPHORUS DETERMINED FROM THE MOLECULAR COHESION

BY ALBERT P. MATHEWS

## *1. The valence of oxygen*

In my paper on the relation of molecular cohesion to molecular weight and valence,<sup>1</sup> in which the number of valences were computed from the value "*a*" of van der Waals' equation, I considered oxygen to be bivalent except in the case of oxygen gas. The question of the quadrivalence of oxygen was not taken up, because I wished to get the main point of the connection of valence and cohesion well established before discussing particulars. But as it is believed by many that oxygen is at times quadrivalent and as this possibility has such an important bearing on theories of association and solubility and, indeed, on the ionizing powers of water, and is also full of importance in physiology, it is interesting to examine the oxygen compounds of that table for evidence in favor of tetravalence.

One atom of oxygen is supposed by Stieglitz, Armstrong, and Goldschmidt<sup>2</sup> to be quadrivalent in the esters. In the first table, therefore, I have incorporated the results of a determination from the molecular cohesion by the usual formula:  $n = a^{3/2} \times 3.2 \times 10^5 / (\text{mol. wt.})$ , of the total number of valences per molecule, and compared it with the number of valences computed if one oxygen is quadrivalent. I have taken only those esters whose critical data were recently very carefully determined by Young. All of these esters associate a little at low temperatures, but their vapors are normal for some degrees below the critical temperature, except possibly that of methyl formate.

<sup>1</sup> Mathews: Jour. Phys. Chem., **17**, 181 (1913).

<sup>2</sup> Zeit. Elektrochemie, **10**, 221 (1904).

TABLE I

Substance	Formula	"a"	Theoretical No. of valences. One oxygen quadrivalent	Number of valences det. from cohesion
Ethyl acetate	$C_4H_8O_2$	0.04076	30	29.9
Ethyl formate	$C_3H_6O_2$	0.03122	24	23.9
Ethyl propionate	$C_5H_{10}O_2$	0.05088	36	36.0
Methyl acetate	$C_3H_6O_2$	0.03206	24	24.8
Methyl butyrate	$C_5H_{10}O_2$	0.05082	36	35.9
Methyl formate	$C_2H_4O_2$	0.02371	18	19.5
Methyl isobutyrate	$C_5H_{10}O_2$	0.04882	36	33.8
Methyl propionate	$C_4H_8O_2$	0.03968	30	28.8
Propyl acetate	$C_5H_{10}O_2$	0.05149	36	36.7
Propyl formate	$C_4H_8O_2$	0.04086	30	29.7

From Table I it is clear that the number of valences computed from the molecular cohesion is very close to the theoretical number, if one of the oxygen atoms is quadrivalent. The only cases in which fewer valences were found were methyl isobutyrate and methyl propionate. In the former, the computation of "a" may not be exactly right as elsewhere pointed out, the isocompounds always giving a value for "a" a little low as compared with the normal. It is, of course, possible that in them the oxygen is bivalent.

Table 2 contains some other oxygen compounds which associate and may be supposed on that account to have quadrivalent oxygen.

In Table 2 the alcohols probably associate somewhat at the critical temperature and acetic acid also; the number of valences, given in the table as computed, were computed with the normal molecular weight and they are, accordingly, too many. It is impossible to determine the total number of valences per molecule by this method for these substances until the average molecular weight has been independently determined at the critical temperature. Of the other substances: acetone, anisol, phenetol, nitrobenzene, acetic anhydride, ethyl diacetate and methyl propyl ketone appear to have tetravalent oxygen. The method, however, is not



TABLE 2

Substance	Formula	"a"	Number of valences computed; one oxygen quadrivalent	No. of valences found
Acetone	$C_3H_6O$	0.02459	22	21.3
Ethyl alcohol	$C_2H_6O$	0.02395	18	25.8 (Assoc.)
Anisol	$C_7H_8O$	0.05645	40	39.8
Methyl alcohol	$CH_4O$	0.01895	12	26.0 (Assoc.)
Phenetol	$C_8H_{10}O$	0.07009	46	48.7
Propyl alcohol	$C_3H_8O$	0.03250	24	31.3 (Assoc.)
Acetic acid	$C_2H_4O_2$	0.03504	18	34.9 (Assoc.)
Nitrobenzene	$C_6H_5NO_2$	0.05968	38	37.9
Acetic anhydride	$C_4H_6O_3$	0.04442	30	29.3
Ethyl diacetate	$C_6H_{10}O_3$	0.06668	42	42.4
Methyl propyl ketone	$C_5H_{10}O$	0.04437	34	34.8
<i>m</i> -Cresol	$C_7H_8O$	0.06254	40	46.0 (Assoc.)

sufficiently accurate to enable this conclusion to be drawn without reservation. The critical data of some of these substances have not been determined with entire accuracy and it is possible, in some, that a very small amount of association may occur at the critical temperature and such association would have the effect of making uncertain the valence determination. With these reservations, however, this method undoubtedly supports the view that oxygen may be tetravalent, and particularly that one atom is tetravalent in the esters.

The method shows oxygen to be bivalent in ether, sulfur dioxide, carbon dioxide and one of the oxygen atoms of the esters.

In oxygen gas, carbon monoxide and nitric oxide, NO, the oxygen is monovalent, if computed from the cohesion. The maximum number of valences in a molecule of oxygen found by this method was two. It must be confessed that it seems unlikely that oxygen is monovalent in the gaseous form, but the critical data are accurately determined and if this method of determining valence is reliable, as it appears to be, there is no escaping the conclusion. The critical data of carbon monoxide should be redetermined, but from those

accepted only two valences can be found in the molecule. Nitric oxide has always been a puzzle, since the oxygen is monovalent, or the nitrogen bivalent. The cohesion shows that there are only two valences, which again means that the oxygen and nitrogen are monovalent.<sup>1</sup>

From its cohesion, then, oxygen appears to be either monovalent, bivalent, or tetravalent.

## 2. Sulfur

Sulfur is generally supposed to be bivalent, but at times to open up two or four residual valences. So far I have not found any compounds with bivalent sulfur, except probably carbonyl sulfide, when the valence is computed from the cohesion. All the sulfur compounds have either quadrivalent or hexavalent sulfur, if the valence is determined by this method. Even sulfuretted hydrogen is no exception to this statement. Table 3 contains the results.

TABLE 3—THE VALENCE OF SUBSTANCES CONTAINING SULFUR

Substance	Formula	“a”	Valences per mol.		Valences per mol. by cohesion
			S = 6	S = 4	
Hydrogen sulfide	H <sub>2</sub> S	0.00890	8	6	7.9
Mercaptane	C <sub>2</sub> H <sub>6</sub> S	0.02497	20	18	20.4
Thiophene	C <sub>4</sub> H <sub>4</sub> S	0.04130	26	24	32.0 (Assoc.)
Sulfur dioxide	SO <sub>2</sub>	0.01349	10	8	7.8
Carbon bisulfide	CS <sub>2</sub>	0.02316	16	12	14.8
Sulfuryl chloride	SO <sub>2</sub> Cl <sub>2</sub>	0.04382	16	14	14.1
Thionyl chloride	SOCl <sub>2</sub>	0.03110	14	12	14.8

<sup>1</sup> A very interesting fact which may be cited in support of the view that oxygen in these three gases is in a state different from the ordinary, and, hence, possibly monovalent, is the following: Oxygen gas and nitric oxide are strongly paramagnetic, and carbon monoxide is far more paramagnetic, or rather far less diamagnetic, than carbon dioxide which contains more oxygen. In all other compounds oxygen is diamagnetic. It is clear that the oxygen in the three gases which this method shows to contain monovalent oxygen has magnetic properties different from oxygen in other oxygen compounds. This fact has not hitherto been explicable. The following figures showing the para- or diamagnetism of different gases I have taken from Auerbach's article on Magnetism in the *Handbuch der Physik*, 5, 274 (1908).

O<sub>2</sub>    NO    CO    Air    C<sub>2</sub>H<sub>4</sub>    CH<sub>4</sub>    CO<sub>2</sub>    N<sub>2</sub>O    N<sub>2</sub>    H<sub>2</sub>  
+4.83; +1.60; -0.009; 1; -0.068; -0.063; -0.033; -0.018; -0.015; -0.002(?)



From Table 2 it appears that sulfur is quadrivalent in sulfur dioxide and sulfuryl chloride; hexavalent in sulfuretted hydrogen, carbon bisulphide, mercaptane, thionyl chloride and probably in thiophene. It is probably bivalent in carbonyl sulphide, but the critical data are uncertain. These results are in agreement with the general idea of the valence of sulfur except in the case of sulfuretted hydrogen, mercaptane, and carbon bisulfide. Thiophene probably associates a little at the critical temperature so that the valence computation is uncertain.

### 3. Nitrogen

Nitrogen is generally supposed to be either mono-, tri- or pentavalent. The cohesion method supports this conclusion. Nitric oxide has been a stumbling block. The results are given in Table 4.

TABLE 4—NUMBER OF VALENCES PER MOLECULE OF NITROGEN COMPOUNDS

Substance	Formula	“a”	Number of valences found from cohesion	Number computed		
				N=1	N=3	N=5
Nitrogen	N <sub>2</sub>	0.0032808	2.0	2	6	10
Nitrous oxide	N <sub>2</sub> O	0.009465	6.2	4	8	
Nitric oxide	NO	0.002570	1.4	2	4	
Nitric dioxide	NO <sub>2</sub>	0.01119	7.6		7	9
Ammonia	NH <sub>3</sub>	0.00844	13.5		6	8 (Assoc.)
Methyl amine	CH <sub>5</sub> N	0.01521	17.9		12	14 (Assoc.)
Dimethyl amine	C <sub>2</sub> H <sub>7</sub> N	0.01922	17.5		18	20
Trimethyl amine	C <sub>3</sub> H <sub>9</sub> N	0.02594	22.7		24	26
Diethyl amine	C <sub>4</sub> H <sub>11</sub> N	0.03625	27.9		30	
Triethyl amine	C <sub>6</sub> H <sub>15</sub> N	0.05415	39.9		42	
Propyl amine	C <sub>3</sub> H <sub>9</sub> N	0.02729	24.5		24	
Dipropyl amine	C <sub>6</sub> H <sub>15</sub> N	0.05835	51.2		52	
Aniline	C <sub>6</sub> H <sub>7</sub> N	0.05157	37.2		34	36
Dimethyl- <i>o</i> -toluidine	C <sub>9</sub> H <sub>13</sub> N	0.08187	51.2		52	54

From the table it appears that nitrogen in nitrogen gas is monovalent. It appears to be also monovalent in nitrous and nitric oxide. Two formulas may be written for nitrous oxide with a total valence of six, *i. e.*,  $\text{N} \begin{array}{c} \diagup \diagdown \\ \text{O} \end{array} \text{N}$  or  $\text{N}-\text{N}=\text{O}$ .

I think the former, with some of the molecule having quadri-valent oxygen, is perhaps the more probable. I have taken the highest possible value of "*a*" for nitrous oxide. In nitric oxide two valences per molecule are the most that can be assigned by this method. This would mean that both elements were monovalent, but as there is other evidence that oxygen is univalent in its elemental form, this formula is not entirely improbable. The valence of nitrogen in nitric dioxide is quite uncertain. In the amines there can be hardly a doubt that it is trivalent, or at times pentavalent with two free valences on the nitrogen. This is the case in ammonia and methyl amine, both of which associate. All of the critical data of the amines were determined by Vincent and Chappuis and I believe their figures are uniformly a little too low. In aniline the nitrogen appears to be pentavalent, but some association occurs. The results are not, then, very satisfactory for these compounds, but they indicate very clearly, however, that nitrogen is either monovalent, trivalent, or pentavalent, as it is supposed to be.

#### 4. Phosphorus

I have found but a single phosphorus compound in which the critical data have been directly determined, although one or two other cases were reported in my paper on the valence of chlorine, in which the critical data had been computed from the surface tension.

In phosphoretted hydrogen the phosphorus is apparently pentavalent, having two free valences. "*a*" is given by Leduc and Sacerdote as 0.00939 and from this the valence of 8.6 is computed. Were the phosphorus pentavalent the total number of valences would be eight. It might be, however, that the phosphorus was heptavalent, but only a few of the molecules had the two pairs of reserve valences open at the same instant. In the chlorine compounds it appeared that the phosphorus was probably heptavalent. I think the only certain conclusion is that the valence is greater than three.



## THE VALENCE OF THE ARGON GROUP AS DETERMINED FROM THE MOLECULAR COHESION

BY ALBERT P. MATHEWS

The valence of the argon group of elements is one of the most interesting problems in chemistry. They are very generally regarded as zero valent, chiefly owing to the position they take in the periodic system between strongly electro-positive and electro-negative, univalent elements. That they are monatomic is undoubted, but they might be monatomic, like mercury vapor, and still have valence. Ramsay<sup>1</sup> made the suggestion, indeed, that they combine into molecules at other than ordinary temperatures. To account for the atomic weight of argon, which computed from the density is 39.9 if the gas is monatomic, he suggested that argon is a mixture of many monatomic molecules with a few diatomic molecules. The ratios of the specific heats as determined is 1.659; whereas if there were 5 percent of diatomic molecules it would be 1.648. The theoretical number, if the gas is entirely monatomic, is 1.667. After discussing this possibility, however, Ramsay says: "But on the whole the presumption is against the hypothesis that argon is a mixture of monatomic and diatomic molecules."

There is some evidence that argon is not entirely lacking in chemical affinity. Berthelot,<sup>2</sup> by the action of the electric discharge on a mixture of argon and benzene vapor, or of argon and carbon bisulphide, produced a brownish deposit on the glass from which argon could be reobtained. Ramsay,<sup>3</sup> in commenting on the absence of the argon lines in the sun's spectrum, suggests, as a reason, that it enters into combination only at high temperatures, these compounds being endothermic; and he cites<sup>4</sup> several observations indicating

<sup>1</sup> Ramsay: "Gases of the Atmosphere," London, p. 231 (1902).

<sup>2</sup> Berthelot: *Comptes rendus*, 120, 581, 660, 1316 (1895); 124, 113 (1897).

<sup>3</sup> Ramsay: *Loc. cit.*, p. 261.

<sup>4</sup> Ramsay: See footnote, p. 538, to article by C. Trenton Cooke: *Zeit. phys. Chem.*, 55, 537 (1906).

a union of argon with zinc, mercury and some other elements. Thus in a Plücker tube the cathode metal disintegrates more rapidly when argon under low pressure is in the tube than when nitrogen is there, and Ramsay interprets this to mean that a volatile compound is formed under the influence of the intense energy at the surface of the electrode and this compound dissociates again setting free the metal, which deposits on the glass. Under his direction C. Trenton Cooke<sup>1</sup> measured the vapor tension of zinc, cadmium, sulfur, mercury and some other metals at high temperatures in the presence of various gases and concluded that the tension of zinc in argon was 12 percent above its tension in nitrogen. Cadmium behaved similarly in helium. Helium seems to be in some kind of a union in fergusonite, and to be capable of feebly uniting with platinum. It may be recalled, also, that the solubility of argon in water is greater than that of helium and nitrogen; and this may be urged as indicating some kind of affinity between water and argon. Chemically, then, these gases, though inert, are not entirely indifferent.

From their action on light,<sup>2</sup> also, a certain argument may be made for their possessing valence. Thus, according to Drude, the dispersion of light in the blue end of the spectrum is due to the valence electrons, and in the red end to the vibrations of the electrically charged atomic groups. If only the valence electrons affect blue light, these gases must also have valence electrons, since they refract light like other gases.

The question whether these elements have valence, or not can be put to a decisive test through their molecular cohesions.<sup>3</sup> There is no question that they possess cohesion, since they can all be liquefied. They behave like all other gases in this respect. Molecular cohesion in all other substances examined is a function of the product of the molecular weight by the number of valences. It has been shown for a

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<sup>1</sup> C. Trenton Cooke: *Loc. cit.*, p. 537.

<sup>2</sup> Cuthbertson, C.: "Refractive Indices of the Elements," *Phil. Trans.*, 204, 323 (1904).

<sup>3</sup> Mathews: *Jour. Phys. Chem.*, 17, 181 (1913).



great number of substances that  $M^2K$ , a factor proportional to " $a$ " of van der Waals' equation, is equal, when expressed in dynes, to  $2.98 \times 10^{-37}$  (mol. wt.  $\times$  No. of valences) $^{2/3}$ . A substance having cohesion cannot, therefore, have zero valence. If it had no valence it could have only gravitational attraction between its monatomic molecules, no cohesive attraction. These gases have cohesion and they must, therefore, have valence.

Their valence,  $n$ , can be calculated from their critical data by the formula: (1)  $n = 0.0043 T_c^3/P_c^{3/2}$  (mol. wt.); or,  $n = a^{3/2} \times 3.2 \times 10^5$ /(mol. wt.); or (2)  $n = 4.21 \times 10^{-5} (V_c T_c)^{3/2}$ /(mol. wt.). The first formula, which is derived from the ordinary formula for computing " $a$ " from the critical temperature and pressure, gives values for " $a$ ," and hence for  $n$ , a little lower than the second formula in the case of simple gases. The second formula computes " $a$ " from the surface tension, as shown in my former paper. In Table 1, I have given both values and I regard the second as the more correct; but as the critical density of not all the gases is known, I have had to rely on the first formula for a comparison. The results given by the two formulas are not widely different.

The valence,  $n$ , together with the critical data<sup>1</sup> used in the calculation are given in Table 1.

TABLE 1—COMPUTATION OF THE AVERAGE NUMBER OF VALENCES PER MOLECULE FROM THE MOLECULAR COHESION

Substances	$T_c$ (Abs.)	$P_c$	$d_c$	Number of valences by formula 1	Number of valences by formula 2
Helium	5.5°	2.75	0.065	0.04	0.07
Helium	8.0	2.75	0.065	0.12	0.12
Neon	61.1	29	—	0.32	—
Argon	150.56	48	0.509	1.12	1.35
Krypton	210.53	54.3	—	1.23	—
Xenon	289.6	58.2	1.155	1.80	1.95

<sup>1</sup> In my preliminary paper, Science, N. S., 36, 6 (1912), in Table I a mistake occurred in the computation of helium. The value 2.90 is wrong. The critical density was taken as 0.015 instead of 0.065.

Before discussing these results a word may be said about the reliability of the critical data. Those of argon and xenon are perhaps the most certain; krypton, neon and helium follow in the order named, helium being least certain. Onnes<sup>1</sup> gives  $5.5^{\circ}$  absolute as the critical temperature of helium, but as this makes helium quite aberrant in several particulars,<sup>2</sup> I have also computed the valence assuming the critical temperature to be  $8^{\circ}$  as suggested by Dewar.<sup>3</sup> The critical data of neon are somewhat uncertain, due in part to the very low critical temperature and in part to the great difficulty in separating the gas completely from helium. A little impurity of the latter gas would have the effect of making  $P_c$  too high.

It is clear from the table that all of these gases possess valence. They are not zero valent as they are supposed by many to be. Furthermore, the average number of valences per molecule is in no case an exact integer, although in argon and xenon it is not far from a whole number. Since these gases have their critical data most accurately determined I at first supposed, as I published in my preliminary paper, that argon was univalent, but slightly associated into diatomic molecules, thus bringing the average number of valences per molecule a little high; and that xenon was divalent. I attributed the deviation of the other gases from univalency, to the inaccuracy of the data. A careful examination of all the facts, however, has led me to abandon this explanation for what seems to me to be a better one, since it explains all the facts.

In the first place, I have not been willing to abandon the idea that valences are indivisible. If we assume, as Lodge<sup>4</sup> suggests, that some of the lines of force from each valence attach themselves to several atoms, or even wander outside

<sup>1</sup> Onnes: *Proc. Amsterdam Acad. Sci.*, 13, 1100 (1911).

<sup>2</sup> Rankin: "On a Relation between Viscosity and Atomic Weight of Inert Gases," *Phil. Mag.*, [6] 21, 45 (1911).

<sup>3</sup> Dewar: Article "Liquid Gases," *Encyclopaedia Britannica*, 16, 749, 11th ed.

<sup>4</sup> Lodge: *Nature*, 70, 176 (1904).



the molecule, and thus split the valence up; or that there are partial valences in the sense of Kauffmann,<sup>1</sup> it seems to me that we might as well abandon the whole valence idea. It entirely loses its usefulness. Again our structural formulas become so indefinite, if the valences are regarded as split up, as to be nearly useless. The explanation which I sought was one which would explain why we appear to have fractional valences in this case, but really do not have them. That my first explanation was wrong, was indicated by the uniformity with which the valence increases from helium to xenon. The deviation, too, from univalency in the case of neon is so great that it lies outside the limits of error. The critical pressure would need to be 14 atmospheres instead of that recorded of 29 atmospheres, in order that neon should have one valence to each molecule. The uncertainty of the critical pressure is far less than this.

I believe the reason that the average number of valences is a fraction in these gases is as follows: All of them are in reality zero valent, so far as their *chief* valences are concerned, but like many, if not all, other elements, they have the power of opening up two residual valences. By their residual valences, therefore, they are all bivalent. These two valences, like most, or all, other residual valences, are of opposite electrical sign, one being positive, the other negative. Not all the atoms have these residual valences open at the same instant, but always some of the atoms have them closed. The molecular cohesion, as I have already pointed out, is not influenced by these valences when they are in a closed or reserve state, or, we might say, withdrawn within the atom; it is only affected by the valences actually extending between atoms, or open and in a state in which they may combine. In xenon nearly all the atoms, or at any rate, 90 percent of them, have the valences open, and the average valence per atom, or molecule, is, therefore, 1.80-1.95; in krypton about 65 percent of the atom have open valences, and 35 percent are closed, so that the average valence is about 1.30; in argon,

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<sup>1</sup> Kauffmann: Ber. chem. Ges. Berlin, 41, 4404 (1908).

about 60 percent are open and 40 percent are closed, the average valence being about 1.20; in neon, 16 percent are open and 84 percent are closed, the average valence being about 0.32; and in helium only about 5 percent of the valences are open, 95 percent being closed, giving an average valence of 0.10. This explains, then, why the elements appear zero valent in the periodic table, since they are zero valent as far as their chief valences are concerned; why, nevertheless, they appear to have some weak chemical affinity, and cohesion; why they refract and disperse light; and also why the average valence is fractional rather than being a whole number.

It also explains more than this. It enables us to understand the easy dissociation of the molecules into atoms. Unlike atoms that are bound together into molecules by their chief valences, no electrical stresses are set up in the argon elements when dissociation into atoms occurs, because each atom having a positive and a negative valence becomes at once electrically neutral. It is well known that compounds formed from residual valences partake of the nature of molecular compounds and break up very easily. Neither their union, nor their dissociation, involves much, if any, energy exchange. Such compounds are often called, indeed, molecular compounds. A double bond of this kind is always a weak bond in any molecule, which easily breaks where the double bond is. Were they univalent their dissociation into atoms would be very hard to understand.

I suppose we may picture the opening of these residual valences in the manner suggested by Sir J. J. Thomson, as being due to a rearrangement of the electrons within the atom so that an excess of negative electricity is temporarily produced in one spot, and of positive at another spot on the surface of the atom. These excesses are the valences.

In closing, it is not without interest to compare the valence numbers computed above from the cohesion, with the refractivity as determined by Cuthbertson.<sup>1</sup> Their refractivities are in the proportion 1, 2, 8, 12 and 19.

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<sup>1</sup> Cuthbertson, C.: *Phil. Trans.*, **204**, 323 (1905).



	Refractivity ( $\mu - 1$ ) $10^6$	Ratio	Valence	Ratio
Helium	36.3	1	0.1	1
Neon	68.7	1.9	0.32	3.2
Argon	284	7.82	1.12	11.2
Krypton	425	11.7	1.23(?)	12.3
Xenon	689	18.98	1.80	18.0

There is a general similarity, but not an identity.

The principal facts and conclusions of the paper are: The molecular cohesion, confirming other properties, shows that the argon group of elements have valence. A computation of the average number of valences per molecule from the molecular cohesion gave the following results: He, 0.1; Ne, 0.32; Ar, 1.12; Kr, 1.23; Xe, 1.80. The valences are apparently fractional, and not whole numbers. The conclusion is that these elements are all zero valent, as far as their chief valences go, but each is divalent in its residual valences. At any one instant of time only a certain proportion of the atoms, varying in the different gases, have their residual valences open, consequently the average number of valences actually open, or active, per molecule is less than two. One residual valence is positive, the other negative; and hence the combining power of the atoms is very weak, since on dissociation an electrically neutral atom is formed, by the saturation within the atom of the oppositely charged valences. This explanation enables us to understand, also, why there is a progressive increase in solubility of these gases in water from helium to xenon if solution be a process involving the union of solvent and solute through their residual valences.

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## A NOTE ON THE STRUCTURE OF ACETYLENE

BY ALBERT P. MATHEWS

From a study of the volume of liquid acetylene, MacIntosh<sup>1</sup> concluded that acetylene was in reality acetylidene since one of the carbon atoms seemed to have the volume of bivalent carbon. He supported this contention also by various chemical arguments. On the other hand, Nef,<sup>2</sup> while showing that the halogen substitution products were in reality acetylidene compounds, believed that acetylene was acetylene and not acetylidene, because it was chemically and physiologically so inert. Nef's pupil, Lawrie,<sup>3</sup> confirmed the acetylidene nature of the bromine and iodine substitution products.

Although it is improbable, for the reasons stated by Nef, that acetylene is acetylidene, the matter may be definitely settled by my method of<sup>4</sup> determining the number of valences in the molecule from the molecular cohesion. If it is acetylene there should be ten valences; if acetylidene, there should be eight, since acetylene does not associate and one carbon atom would be bivalent.

The most recent determination of the critical data of acetylene by Cardoso and Baumé gives  $T_c$ ,  $35.5^\circ \text{C}$ ; and  $P_c$ , 61.6 atmospheres. From these figures the value of " $a$ " of van der Waals' equation calculated by the formula:  $a = 27T_c^2/64 \times 273^2 \times P_c$ , is 0.008745. Computing the number of valences,  $n$  from " $a$ " by the formula:  $n = a^{3/2} \times 3.2 \times$

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<sup>1</sup> MacIntosh: "The Physical Properties of Liquid and Solid Acetylene," Jour. Phys. Chem., **11**, 315 (1907).

<sup>2</sup> Nef: "Ueber das zweiwertige Kohlenstoffatom," Liebig's Ann., **298**, 332 (1897).

<sup>3</sup> Lawrie: "Constitution of Acetylidene Compounds," Am. Chem. Jour., **36**, 487-510 (1906).

<sup>4</sup> Mathews: "The Relation of the Value ' $a$ ' of van der Waals' Equation to Molecular Weight and the Number of Valences of the Molecule," Jour. Phys. Chem., **17**, 181 (1913).



$10^5$ /(Mol. Wt.), we obtain the value 10.06 for  $n$ . There are, therefore, ten valences in the molecule of acetylene; accordingly each carbon has four, each hydrogen one.

Acetylene is, therefore, acetylene, as it is ordinarily written, and not acetylidene.

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## DO MOLECULES ATTRACT COHESIVELY INVERSELY AS THE SQUARE OF THE DISTANCE?

BY ALBERT P. MATHEWS

In a very interesting and valuable recent paper in this journal by Mills,<sup>1</sup> the conclusion was drawn that the cohesive attraction of molecules varied inversely as the square of the distance. Besides this conclusion, which was founded on the interesting discovery that the internal latent heat of vaporization divided by the difference of the cube roots of the densities of the liquid and vapor was a constant, a most valuable part of the paper was the reopening of the question whether the field of molecular attraction is delimited by the surrounding molecules, or whether it owes its small size to the very rapid decrease of the attraction with the distance. This question raised a century ago by Laplace,<sup>2</sup> was answered by him in the latter sense without any convincing reason for his conclusion, and has not been reopened since, the opinion being almost universal that the shortness of the radius of action is due to the attraction diminishing with the distance at a rate far more rapid than the square; the fourth, fifth, seventh and even higher powers having been suggested. In thus reopening the question Mills has rendered a valuable service. That his conclusion is correct, that the molecular field is delimited by the surrounding molecules, is clearly indicated by Einstein's<sup>3</sup> calculation of the radius of action, showing that the radius is proportional to the distance between the molecular centers. The conclusion that the attraction is inversely as the square of the distance, however, I believe to be erroneous for the reasons which will be presented in this paper.

<sup>1</sup> Mills: Jour. Phys. Chem., 15, 417 (1911).

<sup>2</sup> Laplace: "Traité de mécanique céleste," Supp. au Livre 10, p. 351.

<sup>3</sup> Einstein: Drude's Ann., 34, 165 (1911).



Mills<sup>1</sup> discovered the empirical relationship that the quotient of the internal latent heat of vaporization divided by the difference of the cube roots of the densities of the liquid and vapor was a constant, except in the neighborhood of the critical temperature. If  $L$  is the total latent heat, and  $E$  is the part of it used in doing external work, then  $L - E$  would be the part of the heat used in doing internal work. He found that  $(L - E)/(d_1^{1/3} - D_v^{1/3}) = \mu'$ . He assumed that this internal heat was all used in overcoming molecular cohesion, and he ascribed the fall of the constant near the critical temperature to the inaccuracy of the data. He then reasoned that since for  $d_1^{1/3} - D_v^{1/3}$  the expression  $1/V_1^{1/3} - 1/V_v^{1/3}$  might be substituted, the molecules must attract each other inversely as the square of the distance; since it is only on such a supposition that the difference in potential energy of the molecules in the liquid and the vapor can be given by an expression of this kind. The similarity of his expression:  $L - E = \mu'(1/V_1^{1/3} - 1/V_v^{1/3})$  to Helmholtz's formula for the heat given off by the contraction of the sun seemed significant, the Helmholtz formula being  $W = \frac{3}{8}M^2K^2(1/R - 1/CR)$ . From this similarity Mills reasoned that molecular attraction, like gravitational, must follow the inverse square law. Since it is impossible that molecules should attract each other cohesively according to this law, if the cohesive attraction penetrated matter, he concluded that cohesion did not penetrate matter, but was delimited by the surrounding molecules.

There is, however, another relationship expressing the latent heat consumed in overcoming molecular cohesion or the internal pressure, which has been given by van der Waals and is derived from his expression for cohesive pressure of  $a/V^2$ . This relationship is:  $L - E = a(1/V_1 - 1/V_v)^2$  where

<sup>1</sup> Mills: Jour. Phys. Chem., 15, 417 (1911); Comptes rendus, 153, 193 (1911); Phil. Mag., [6] 22, 84 (1911); [6] 23, 484 (1912).

<sup>2</sup> This formula should, in my opinion, be written:  $L - E - X = a(1/V_1 - 1/V_v)^2$  where  $X$  represents heat used in any other internal work than the separation of the molecules. See van der Waals: "Condensation of Gases," Encyclo. Britannica, xi edition. Also Sutherland: Phil. Mag., [5] 22, 83 (1886).

TABLE I—COMPARISON OF MILLS' CONSTANT "C" WITH "C'"

$$C = (L - E) / (\sqrt[3]{d} - \sqrt[3]{D}); C' = 3N^2MK / Wt^{1/2} V_c^{2/3}$$

Ethyl acetate

Methyl butyrate

Temperature	C	C'	Temperature	C	C'
100°	3.879 × 10 <sup>11</sup>		100°	3.681 × 10 <sup>11</sup>	
200	3.915		200	3.643	
270	3.893		220	3.596	
280	3.416		240	3.461	
281.3	Critical	3.442 × 10 <sup>11</sup>	247	3.324	
			249	3.329	
			250.1	Critical	3.222 × 10 <sup>11</sup>
Diisobutyl					
100°	4.168		Ether		
180	4.114			3.259	
200	4.148			3.216	
260	4.265			3.208	
274	4.113			3.158	
276.8	Critical	3.697		3.017	
				2.862	
				Critical	3.061



Methyl acetate		Methyl propionate	
100°	3.362	100	3.658
200	3.350	200	3.664
220	3.250	220	3.659
230	3.108	250	3.459
233	2.989	256	3.273
233.7	Critical	257.4	Critical
	3.064		3.251
Normal pentane		Benzene	
0°	3.354	80°	3.564
40	3.295	100	3.549
100	3.314	140	3.531
180	3.289	180	3.595
195	3.093	200	3.593
197.1	2.967	220	3.613
197.15	2.984	280	3.513
197.2	Critical	288.5	Critical
	3.181		3.471

"*a*" is van der Waals' constant. According to Sutherland<sup>1</sup> the latter expression indicates that the attraction of the molecules is inversely as the fourth power; whereas Mills has interpreted the former as meaning that it is inversely as the square.

To show how constant Mills' constant is, I have given, in Table 1, the results of the calculations by his formula of a number of substances from Young's data. The figures represent ergs for gram molecular quantities. It will be seen that the constancy is good for a considerable range of temperature, but that in all cases there is a more or less pronounced drop close to the critical temperature, and in some, as in ether and ethyl acetate, there is a pretty steady fall in the constant throughout. The fall near the critical temperature might be ascribed to errors of observation, or calculation. There is no doubt, therefore, that for most substances the expression  $(L - E)/({}^3\sqrt{d_1} - {}^3\sqrt{D_v})$  closely approximates a constant except near the critical temperature, as Mills has pointed out.

The conclusion that this relationship shows that the molecules attract inversely as the square of the distance is, I believe, sound, if the premise is correct. The premise, or assumption, is that the internal latent heat of vaporization, or  $L - E$ , represents only the work done in separating the molecules against their molecular cohesion. While Mills<sup>1</sup> states in a recent paper that not all the internal heat may be used in doing this work, and attempts to show that this is not incompatible with this conclusion, the conclusion nevertheless depends on the assumption that it is so used and that there is no change in the internal energy of the molecules on passing from the liquid to the vapor. It is clear that if this premise be not true, then the conclusion does not follow.

This premise I believe to be certainly erroneous. It could only be true if the molecules remained of the same

<sup>1</sup> Mills: *Phil. Mag.*, [6] 22, 97 (1911); 23, 499 (1912).



size in liquid and vapor, or do not in other ways gain energy.<sup>1</sup> I believe the internal latent heat of vaporization consists of at least three parts, not two as is often stated, these three parts are: (1) the heat consumed in expanding against external pressure, or  $E$ ; (2), the heat consumed in overcoming molecular cohesion, or  $A$ ; (3), heat consumed in increasing internal molecular potential energy by expanding the molecule, or increasing its energy of rotation, or  $I$ . This last factor is often overlooked. If  $L$  is the total latent heat of vaporization the expression should be:  $L - E - I = A$ . And if molecules attract inversely as the square of the distance we should have  $(L - E - I)/({}^3\sqrt{d_e} - {}^3\sqrt{D_v}) = \mu'$ .

There are two principal reasons why it cannot be assumed that all the internal latent heat of vaporization goes to increasing the potential energy by separating the molecules against the force of their molecular cohesions. The first of these reasons is that the value " $b$ ," of van der Waals' equation, has to be taken larger in the vapor than in the liquid for some distance below the critical point. And there are good reasons for thinking that " $b$ " represents the real volume of the molecules. The second reason is the value  $a/V^2$  representing cohesion in van der Waals' equation. A third reason has been given by Tyrer.

To show that the molecules actually do expand in passing from the liquid to the vapor, I have calculated the value of " $b$ " for pentane, and benzene using Young's data. I have also calculated several others of his substances, but as the result is similar in them to that in these two, I give only the latter in Table 2, which shows the value of  $b$  in cc in the liquid and vapor for gram molecular quantities  $b = V - RT/(P + a/V^2)$ .<sup>2</sup>

<sup>1</sup> A similar objection to Mills' conclusion has been raised by Professor Tyrer: *Phil. Mag.*, [6] 23, 112 (1912).

<sup>2</sup> Since sending this paper to the publisher I have found that the value of " $a$ " is larger than assumed here. This change requires  $b_e$  and  $b_v$  both to be larger. At  $150^\circ$   $b_v$  should be about 127 cc and at  $40^\circ$  only —14. The relation between  $b_e$  and  $b_v$  is not greatly changed, but the difference between them becomes greater.

TABLE 2  
Pentane

$t$	$b_1$	$b_v$	$V_v$	P (At)	$a/V_v^2$
40°	100.6	—140	21,420	1.15	0.04
100	106.5	— 64.1	4,428.00	5.80	1.09
150	114.6	79.9	1,512.60	15.54	8.72
180	124.2	140.3	790.05	25.46	31.96
190	130.8	151.9	567.36	29.61	61.90
195	137.2	155.2	447.5	31.91	99.55
197	144.5	153.3	359.10	32.90	154.70
197.2	149.4	149.4	309.94	33.03	207.50 (critical)

Benzene

$t$	$b_e$	$b_v$	$V_v$
80°	82.3	70	28,570
120	84.6	—170	10,160
160	87.4	— 22	5,428
200	90.8	52	2,200
240	96.2	89	1,093
260	100.5	110.3	751.7
270	103.6	120	606
280	108.2	127	470
288.5	123.8	123.8	256.2

Table 2 shows that, in pentane,  $b_v$  is larger than  $b_1$  from the critical temperature to about 160°. Below this point  $v_v$  falls rapidly and apparently soon becomes negative. The reason for this apparent fall is undoubtedly the association, or quasi-association, occurring in the vapor as the temperature falls, as van der Waals suggests, the result being that the number of the molecules in the space does not remain constant and hence  $R$  does not remain constant. The effect of reducing  $R$  to its real value, were we able to correct for the association, would be to make  $b_v$  larger. In benzene  $b_v$  falls below  $b_1$  sooner than in pentane, from which we may infer that the association in benzene is a little larger than in pentane. The apparently negative value of  $b_v$  is found closer to the critical temperature in the esters which are known to associate slightly. Since association produces an



apparent decrease in  $b_v$ , it is practically certain that the differences between  $b_1$  and  $b_v$  are actually larger than those indicated. The main fact is then established that  $b_v$  is actually larger than  $b_1$  for some degrees below the critical point in spite of the association which tends to mask the actual molecular expansion and which, at lower temperatures, conceals it entirely.

It will be seen, also, that, as one would expect,  $b_v$  actually decreases close to the critical temperature, owing to the compression of the molecules due to the great increase in internal and external pressure. This increase of pressure ( $P + a/V^2$ ) is indicated in Table 2 in the case of pentane, the pressures being given in atmospheres per sq. cm.

It is of interest, in this connection, to compute what is possibly the real value of  $b_v$ , making van der Waals' assumption that in the vapor, at low temperatures,  $b_v$  is equal to  $2b_1$ . Supposing that this is the case at absolute zero we may write the rectilinear diameter formula:  $b_1 + b_v = b_c ((3T_c + T)/2T_c)$ . This assumes that at absolute zero the molecules are so compressed that in the solid their volume is one-half of what it would be in the vapor at the same temperature, and that the volume of the vapor molecules at absolute zero is that of  $b_c$ .  $b_c$  is very nearly  $V_c/2$ . Table 3  $b_v = b_c((3T_c + T)/2T_c) - b_1$ .

TABLE 3  
Pentane

$t$	$b_1$	$b_v$ (cal)	$b_v$ (taken from Table 2)
40°	100.6	173.2	—140
100	106.5	176.9	— 64.1
150	114.6	176.7	79.9
180	124.3	171.9	140.3
190	130.8	166.8	151.9
195	137.2	161.3	155.2
197	144.5	154.3	153.3
197.2	149.4	149.4	149.4

The parabola represented by these figures is given in Fig. 1. If there is any association in the liquid the effect

of correcting for it would be to reduce the value of  $b_v$  and increase  $b_1$  and so to make the parabola flatter. I have computed  $b_1$  assuming that there is no association in the liquid. The increase of  $b_v$  with the temperature is seen to be very slight. From absolute zero to the maximum value at  $100^\circ$  the increase is at the rate of 0.074 cc per degree for gram mol quantities. On the other hand " $b$ " changes markedly with the pressure.

There are several reasons for believing that " $b$ " is the real volume of the molecules and not four times the volume as was originally suggested. One is that " $b$ " at the critical

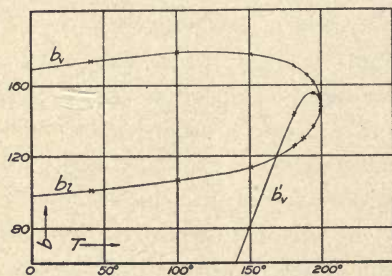


Fig. 1—Volumes of molecules ( $b$  for gram mol quantities in cc.) for pentane.  $b_1$  calculated from formula  $b_1 = V_1 - RT/(P + a/V_1^2)$  assuming no association;  $b_v$  calculated by formula:  $b_v = b_c((3T_c + T)/2T_c)) b_1$ ;  $b_v'$  apparent volume of  $b_v$  by formula:  $b_v' = V_v RT/(P + a/V_v^2)$  assuming no association.

temperature is very nearly  $V_c/2$  and this is just twice the volume at absolute zero. It is unlikely that the molecules do not expand in passing from absolute zero to the critical temperature, since at the former temperature they are under a pressure of 3572 atmospheres in pentane, whereas at the critical temperature the pressure is only 240 atmospheres. It would take very little separation of the atoms to double the volume.

The latent heat shows, also, that heat is absorbed by the molecule roughly proportional to the number of atoms in the molecule. This would mean that the atoms vibrated (or expanded) and they must hence take up more space as the vigor of vibration increases. This would seem to be sufficient



to account for doubling the volume of  $b$  between  $0^\circ$  Abs. and  $T_c$ . Van der Waals<sup>1</sup> himself only assumed the constancy of the molecular volume " $b$ " for simplicity, and has now definitely adopted the idea of a change in volume of the molecules. He has obtained, by making certain assumptions, the following expression for the change of " $b$ ," the molecular volume:  $(b - b_0)/(V - b) = 1 - (b - b_0)^2/(b_g - b_0)^2$ . In this equation  $b_g$  and  $b_0$  are the limiting values of  $b$ ;  $b_g$  at low vapor pressures, and  $b_0$  under high pressure; the actual value under any temperature and pressure is " $b$ ." Van der Waals assumes that in liquids at low temperatures,  $b_g = 2b_0$ . Both probability and direct observation lead, therefore, to the conclusion that the molecules expand on passing from the liquid to the vapor state.

It is clear that if the molecules do thus expand against the great force of atomic affinity, or intra-molecular cohesion, some heat must be absorbed. The quantity thus absorbed will probably be greatest at low temperatures, where there is a maximum difference in cohesive pressure between the liquid and the vapor, and will diminish rapidly near the critical temperature, since the volumes of the molecules in the two states approach each other and become equal at the critical temperature. As we near the critical temperature, therefore, the value of  $I$  will become very small, and the equation  $I - E = A$  will become very nearly true.

The second reason why the internal latent heat of vaporization can not be assumed to go altogether to increasing the distance between the molecules is the fact that the internal pressure, the cohesive pressure, is inversely proportional to the square of the volume. For, assuming as before that all the latent heat goes toward separating the molecules, if  $a/V^2$  is the cohesive pressure per unit surface then the cohesive energy in the liquid will be  $a/V_l$ ; and in the vapor,  $a/V_v$ ; and the difference in their cohesive energies will be

<sup>1</sup> Van der Waals: "The Liquid State and the Equation of Condition," Proc. Roy. Acad. Sci. Amsterdam (English Translation), 6, 123 (1903).

$a(1/V_1 - 1/V_v)$ . By our assumption this difference in energy must be equal to  $L - E$ . Hence  $(L - E)/(1/V_1 - 1/V_v)$  must equal " $a$ ." We come, therefore, to an expression different from Mills and one incompatible with it. Since it is certain that the cohesive pressure varies at least approximately inversely as the square of the volume this expression must be the correct expression, if  $L - E$  represents only heat consumed in overcoming cohesion. As a matter of fact  $(L - E)/(1/V_e - 1/V_v)$  does not equal a constant, hence our assumption must be wrong. But if the assumption is wrong then the fact that  $(L - E)/(1/V_1^{1/3} - 1/V_v^{1/3})$  happens to equal a constant can not be adduced as evidence that molecules attract inversely as the square of the distance.

I think therefore, that Mills' empirical expression,  $L - E = \mu'(1/V_1^{1/3} - 1/V_v^{1/3})$  does not mean, as he supposed, that the work done in overcoming molecular cohesion from the volume  $V_1$  to the volume  $V_v$  was equal to  $\mu'(1/V_1^{1/3} - 1/V_v^{1/3})$ , but that the total internal latent heat, *i. e.*, that used in overcoming molecular cohesion as well as that absorbed in the expansion of the molecules, or in doing other work is equal to this expression.

That Mills' expression,  $\mu'(1/V_1^{1/3} - 1/V_v^{1/3})$ , does not represent the work done in overcoming molecular cohesion may be shown, also, if the attempt is made to deduce the formula on this basis, assuming the attraction to vary inversely as the square of the distance. A value is obtained for  $\mu'$  widely different from that found. Mills realized this difficulty and tried to avoid it by assuming that the law that matter attracted itself as the product of the masses was incorrect.

I will make the simplest possible assumptions. If the molecules are assumed to be cubical in shape, to lie a mean distance apart and the lines of attractive force to run perpendicularly from each face of the cube in three directions of space and to end upon the six surrounding molecules, but not to penetrate them; and if the molecules attract with a force varying inversely as the square of the distance between



the centers and directly as the product of the cohesive masses  $M$ , then the attraction of two molecules would be  $M^2K/v^{2/3}$ . The pressure per square cm would be  $M^2K/v^{4/3}$ . Since the attraction goes but a single molecular diameter we may multiply the numerator and denominator by  $N^{4/3}$ , where  $N$  is the number of molecules in the mass which will make  $N^{4/3}M^2K/V^{4/3}$ . It is obvious that this expression cannot be true, for the cohesion varies inversely as the square of the volume, and not as the  $4/3d$  power. Assuming, however, that it is correct we would have, as the difference in the cohesive energies in the liquid and vapor, the expression:  $N^{4/3}M^2K(1/V_1^{1/3} - 1/V_v^{1/3})$ . Or changing to density  $N^{4/3}M^2K(d_1^{1/3} - D_v^{1/3})/Wt^{1/3}$ . Hence  $L - E$  should equal this expression, and  $(L - E)/(d_1^{1/3} - D_v^{1/3}) = N^{4/3}M^2K/Wt^{1/3} = \mu'$ . This last expression can be tested, since  $M^2K$  can be easily computed from van der Waals " $a$ " by dividing it by  $N^2$ , the square of the number of molecules in the volume  $V$ , or  $Wt$ ; and  $\mu'$  is given by Mills. The two values are not of the same order of magnitude. For example in pentane,  $\mu'$  is 110, whereas  $N^{4/3}M^2K/Wt^{1/3}$  for 1 gram is  $2.214 \times 10^{-13}$  calories. A constant very like  $\mu'$  is obtained, however, if the foregoing constant is divided by  $V_c^{2/3}/3N^{2/3}$ . This changes it to the expression  $3N^2M^2K/V_c^{2/3}Wt^{1/3}$ . This would give the value 102 for pentane. How closely this constant agrees with Mills is shown in Table 1.  $N^2M^2K$  is equal to " $a$ ."

The constant cannot be deduced, therefore, by the assumptions we have made, one of them being that molecules attract each other inversely as the square of the distance, but it is necessary to divide the theoretical constant by  $V_c^{2/3}/3N^{2/3}$  to get that found. This however, has the effect of changing the equation, near the critical temperature, to the form:  $L - E = 3a(1/V_1 - 1/V_v)$  which is almost identical with van der Waals.

This argument will perhaps be still more convincing if it be turned around. Let us suppose Mills' contention is correct and the internal latent heat represents only heat used in overcoming cohesion, then  $\mu'/V_1^{1/3}$  is the cohesive energy

<sup>1</sup> Mathews: Jour. Phys. Chem., 17, 154 (1913).



TABLE 4—(Continued)

Diisopropyl			Ethyl acetate		
Temp.	$\frac{L-E}{d-D}$	$\frac{N^2M^2}{K/Wt}$	Temp.	$\frac{L-E}{d-D}$	$\frac{N^2M^2}{K/Wt}$
100°	$3.808 \times 10^{11}$	2.877	100°	3.462	2.357
200	3.291		200	3.016	
220	3.039		220	2.873	
225	2.895		240	2.645	
227.35	Critical		247	2.482	
			249	2.472	
			250.1	Critical	
Methyl propionate			Methyl formate		
100°	$3.417 \times 10^{11}$	2.353	100°	$2.481 \times 10^{11}$	1.791
200	3.030		200	2.015	
220	2.931		210	1.842	
250	2.598		213	1.747	
256	2.396		213.5	1.710	
257.4	Critical		214	Critical	
Pentane normal			Stannic chloride		
0°	$4.013 \times 10^{11}$	2.806	100°	$1.573 \times 10^{11}$	1.106
40	3.807		200	1.413	
100	3.558		240	1.358	
180	3.085		260	1.322	
195	2.769		280	1.280	
197.1	2.621		318.7	Critical	
197.15	2.637				
197.2	Critical				
Ethyl propionate			Propyl acetate		
100°	$3.901 \times 10^{11}$	2.552	100°	3.929	2.575
200	3.385		200	3.507	
270	2.825		270	2.860	
272.8	Critical		275	2.721	
			276.2	Critical	
Benzene			Ethyl formate		
80°	$3.486 \times 10^{11}$	2.556	100°	$2.968 \times 10^{11}$	2.121
100	3.413		200	2.532	
140	3.272		230	2.201	
180	3.197		234	2.087	
200	3.118		235.3	Critical	
220	3.052				
280	2.676				
288.5	Critical				

TABLE 4—(Continued)

Isopentane			Methyl butyrate		
Temp.	$\frac{L-E}{d-D}$	$\frac{N^2M^2}{K/Wt}$	Temp.	$\frac{L-E}{d-D}$	$\frac{N^2M^2}{K/Wt}$
20°	$3.694 \times 10^{11}$				
100	3.367		100°	3.776	
160	3.067		200	3.429	
185	2.694		270	3.033	
187	2.616		280	2.573	
187.4	2.559		281.3	Critical	2.559
187.8	Critical	2.726			
Heptane			Methyl isobutyrate		
0°	5.045		100°	3.675	
100	4.730		200	3.256	
180	4.314		265	2.687	
220	4.114		266.5	2.620	
260	3.668		267.55	Critical	2.488
266	3.428				
266.5	3.343				
266.85	Critical	3.187			
Ether			Carbon tetrachloride		
0°	3.596		0°	1.907	
20	3.481		80	1.823	
100	3.160		100	1.792	
180	2.708		120	1.756	
190	2.516		180	1.661	
193	2.332		200	1.590	
193.4	Critical	2.468	260	1.520	
			280	1.419	
			283.15	Critical	1.383
Hexane			Fluorbenzene		
0°	4.504		80°	3.096	
80	4.217		200	2.672	
100	4.128		286.55	Critical	2.391
180	3.684				
200	3.568				
234	3.004				
234.8	Critical	3.003			

It is of interest to see how large the amount of heat (I) is which is absorbed intramolecularly on passing from liquid to vapor. Table 5 contains the calculations for I of 1 gram of hexane.  $I = L - E - a(1/V_1 - 1/V_v)$  gram calories.



TABLE 5—HEXANE

$t$	$L - E$	$I$
0°	84.68	28.21 Calories
60	73.59	21.88
80	70.04	20.17
100	65.82	18.06
180	44.30	8.19
200	37.00	5.86
230	16.98	0.93
234	8.98	0.00
234.5	0.00	0.00 Critical

The intramolecular heat ( $I$ ) absorbed is obviously considerable.<sup>1</sup> At corresponding temperatures the amount of intramolecular latent heat is for gram mol quantities, greater, the larger the number of atoms in the molecule as may be seen in Table 4.

The conclusion is, therefore, that van der Waals' relationship, *i. e.*,  $L - E = a(1/V_1 - 1/V_v)$  is correct close to the critical temperature, but should be changed to the form  $L - E - I = a(1/V_1 - 1/V_v)$ , where  $I$  is the heat made latent intramolecularly by expansion of the molecules, or increasing rotation, in passing from the liquid to the vapor; and that while Mills relationship:  $L - E = \mu'(1/V_1^{1/3} - 1/V_v^{1/3})$  is empirically true except, possibly, near the critical temperature, yet since  $L - E$  does not represent solely the increase in potential energy due to the separation of the molecules, the inference he has drawn from it, that molecules attract inversely as the square of the distance, is not justified. On the other hand, the correct expression for the increase of molecular potential energy,  $a/(1/V_1 - 1/V_v)$ , is obtained very simply if the molecules attract inversely as the fourth power of the distance, as Sutherland has shown.

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<sup>1</sup> "a" should be larger and the figures in column 3 should be somewhat smaller. See footnote p. 525.





# THE SIGNIFICANCE OF THE RELATIONSHIP BETWEEN MOLECULAR COHESION AND THE PRODUCT OF THE MOLECULAR WEIGHT AND THE NUMBER OF VALENCES

BY ALBERT P. MATHEWS

In the preceding papers<sup>1</sup> of this series I have shown that the value of "*a*" of van der Waals, representing molecular cohesion, or the value  $M^2K$  which is the factor "*a*" for a single molecule, is proportional to the two-thirds power of the product of the molecular weight by the number of valences of the molecule.  $M^2K$  was found to be equal, when expressed in absolute units, to  $2.98 \times 10^{-37}$  (Mol. Wt.  $\times$  No. of Val.)<sup>2/3</sup>.

In this paper I shall discuss the theoretical bearing of the relationship of cohesion to these molecular properties.

Attempts have been made by others to correlate cohesion, or "*a*," with molecular weight and the number of valences, but with very partial success. Sutherland<sup>2</sup> at first supposed the molecular attraction to be proportional to the product of the gravitational masses of the molecules. This he found would not do, and in his later papers he stated that the gravitational mass of a molecule did not enter into the expression "*a*." Amagat,<sup>3</sup> also, recently revived the idea that gravitational mass plays a role in cohesion and suggested that  $a/V^2$  ought to be proportional to the square of the molecular mass. This, however, he did not find to be the case. Leduc<sup>4</sup> has recently confirmed, in part, this view of Amagat's for gases of similar molecular composition, when taken under the same volume and at corresponding temperatures. Kleeman,<sup>5</sup> also has tried to find a relationship between "*a*" and gravita-

<sup>1</sup> Mathews: Jour. Phys. Chem., 17, 154 (1913).

<sup>2</sup> Sutherland: Phil. Mag., [5] 27, 305 (1889); [6] 4, 632 (1902).

<sup>3</sup> Amagat: "Pression interne des fluides," Journal de Physique, [4] 8, 617 (1909).

<sup>4</sup> Leduc: Comptes rendus, 153, 179 (1911).

<sup>5</sup> Kleeman: Phil. Mag., [6] 19, 783, 840-847 (1910).

tional mass, and states that the cohesive attraction of two molecules is proportional to the product of the two sums of the square roots of the atomic weights of the atoms of the molecules. This relationship, however, is of very limited applicability, if indeed, it correctly expresses the cohesion of any.

As regards valence, I can find but one other suggestion, that of Sutherland.<sup>1</sup> He showed that the number of equivalents, or valences, in simple substances, such as sodium chloride, influenced the value of their cohesion. He was unable to establish this relationship for more complex bodies. Nevertheless he assumed that it existed in them and correctly surmised from it the relationship between cohesion and chemical affinity, and adduced it as evidence of the electrostatic or magnetic nature of cohesion. "*a*" was made proportional to the square root of the valence.

The relationship between cohesion and the properties of molecular weight and the number of valences can be interpreted best by Sir J. J. Thomson's theory of the electrical constitution of matter and valence, and, so far as I can see, on no other hypothesis. It speaks, therefore, for the electrostatic, or electro-magnetic theory of cohesion, and, in my opinion, for the latter.

The relation,  $M^2K = (f)(\text{Val}^{\frac{2}{3}}/\text{Mol. Wt.})^{\frac{2}{3}}$ , seems at first peculiar. It is odd that the valence of an atom should be of as much importance in cohesion as the weight of the atom; it is a relationship which one would not have anticipated. The significance of this fact, if I am not mistaken, is that the electron couples constituting the molecules are of two kinds, namely, those of the atoms themselves, which added together presumably give the molecular weight; and the valence electrons, which differ from the others so that they cannot be added to them. Hence the formula is not  $M^2K = (f)(\text{Wt.} + \text{Val.})$ , the cohesion being proportional to the sum; but the mass of cohesion is proportional to the cube root of each of

<sup>1</sup> Sutherland: *Phil. Mag.*, [6] 4, 632 (1902).



these kinds of electrons and so is proportional to the cube root of their product. The valence electrons are probably more labile, more easily removed and replaced. They have a different degree of liberty and they cannot be summed with the atomic.

The formula thus confirms the correctness of Drude's promise that the electrons of the valences differ in their properties from the electrons of the atoms. He concluded that only the valence electrons would be sufficiently free to vibrate synchronously with light and hence these electrons must be particularly concerned in the refraction and dispersion of light. Drude's<sup>1</sup> suggestion of electrons of different degrees of liberty confirmed, as it was, by experiments showing a relation between valence and dispersion, is thus confirmed also from the wholly different field of cohesion.

A still more interesting conclusion may be drawn from this relationship, namely, that a neutral, uncharged atom having no valence will have no cohesion. Since it will have no chemical affinity either, if chemical affinity is, as it appears to be, of an electrical nature, it is thus seen that a close relation must exist between chemical affinity and cohesion. Such neutral atoms will presumably still have gravitational attraction. A free electrical charge on the atom is, therefore, necessary for cohesion, but not for gravitation. Furthermore, the cohesive effect is the same whether the charge be positive or negative; and it is proportional to the number of charges. The formula shows, also, that the effect of a free charge on any atom is proportional to the weight of the atom; that is, the effect of the valence charge is multiplied, as it were, by the number of electron couples in the atom; and the effect of the total number of valence charges in the molecule is multiplied by the whole number of atomic electron couples in the molecule. Just how such an effect could be produced, and why the attraction, or cohesive mass, should ultimately prove to be proportional to a linear function (the cube root) of the product

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<sup>1</sup> Drude: *Annalen der Physik.*, [4] 14, 677 (1904).

of the number of valences by the molecular weight, I do not see.

It appears, then, that refraction, dispersion and cohesion all involve the valence electrons, but the connection between cohesion and valence is far closer and simpler than the other relationships appear to be. The relationship of valence to light is necessarily a less direct one, refraction depending on the rate of vibration of the electron. It is said<sup>1</sup> that if the natural period of the molecule (electron) is slightly less than the frequency of a light wave the light will be accelerated; if greater, retarded. It is evident that in dispersion other properties of the electrons than number come into play, and, hence, the relationship between dispersion and number is not so simple and direct. Double bonds, neighboring groups, etc., influence the periods of the electrons and so influence the dispersive power; whereas these factors appear to play no important part in cohesion.

The relation between the refraction of light of one wave length and the valence number is still less direct than between dispersion and valence, but still a general relation exists which for substances of the same type is rather uniform, as shown by Traube<sup>2</sup> for many liquids and by Cuthbertson<sup>3</sup> for several gases.

Another very interesting fact correlating the refractive and cohesive properties of matter is the resemblance between the constant "K" of the Ketteler dispersion formula and the value  $M^2K$  of cohesion. Thus with the Ketteler formula  $n^2 = a^2 - K\lambda^2 + D\lambda^2_v/(\lambda^2 - \lambda^2_v)$  the constant "K," Drude found, could be computed with a fair approximation, in some cases at any rate, from the sum of the valences, the molecular weight and the density, and this result was confirmed by Erfle.<sup>4</sup> This constant "K," therefore, contains at least

<sup>1</sup> Cotter, J. R.: "Dispersion," *Encyclo. Brit.*, 11th edition, 8, 317.

<sup>2</sup> Traube: *Ber. chem. Ges. Berlin*, 40, 130 (1907).

<sup>3</sup> Cuthbertson: *Proc. Roy. Soc.*, 83A (1909-1910); *Phil. Mag.*, [6] 21, 69 (1911); *Phil. Trans.*, 204, 323 (1905); 207, 135 (1907).

<sup>4</sup> Erfle: "Optische Eigenschaften und Elektronen Theorie." *Annalen der Physik*, [4] 24 (1907).



sometimes the same factors as the value  $a/V^2$  of van der Waals' equation. I have not, however, attempted to establish any closer connection between them.<sup>1</sup>

We conclude, therefore, that while cohesion and refractivity are both dependent on a common factor, namely the valence electrons, and possibly upon the molecular weight, the connection between them is not direct, but indirect; and while cohesion and refraction, or dispersion, often parallel each other, they, at other times, diverge considerably since other factors enter into refraction.

It is not without interest to recall as an example of the perspicacity of genius, that Laplace<sup>2</sup> long ago foretold a connection between these properties. Writing in 1805 of the formula of capillarity which, as will be remembered, contained two terms, one  $K$ , representing molecular cohesion, or van der Waals' expression  $a/v^2$ ; the other,  $H$ , the capillary constant, Laplace says (p. 351): "I saw that this action (pressure) is smaller or larger than if the surface is plane; smaller, if the surface is concave; larger, if it is convex. Its analytical expression is composed of two terms: the first ( $K$ ), much larger than the second, expresses the action of the mass terminated by a plane surface; and I think from this term depends the suspension of mercury in a barometer tube at a height two to three times greater than that due to atmospheric pressure, the refractive powers of diaphanous bodies, the cohesion, and in general, chemical affinity; the second term expresses the part of the action due to the sphericity of the surface." And again (p. 362): "The function,  $K$ , is analogous to that I have designated by the same letter in the refraction of light."

But of even greater interest and more fundamental importance than the relation between the optical and the cohesive properties, which is now understandable since both

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<sup>1</sup> See also Natanson: *Bull. de l'Acad. des Sci. de Cracovie*, 1907, April p. 316 for the relation of refraction and valence.

<sup>2</sup> Laplace: *Sur l'action capillaire. Oeuvres. Supp. Liv. X, Traité de Mécanique Céleste*, p. 351.

involve the number of valence electrons, is the relation between the magnetic and cohesive properties, since here we touch, I think, the very kernel of the problem of the nature of cohesion.

The connection between the magnetic properties and cohesion is brought out very clearly, in an empirical way, by Pascal's<sup>1</sup> investigations on the relation between magnetic susceptibility and the molecular properties. In a series of papers Pascal has shown that there is a remarkable connection between the specific susceptibility of diamagnetic elements and the atomic weights and valences. Thus if elements of the same family having the same valence are arranged in their order of increasing specific susceptibility, they are in the order of their atomic weights; and, on the other hand, a close dependence on valence may be observed. In elements of nearly the same weight but of different valence numbers, the atomic magnetic susceptibility,  $X_a$ , increases with the number of valences. An empirical relationship was found between them,  $X_a = -10^{-7}e^{\alpha + \beta a}$  where  $a$  is about 2.1,  $\beta$  about 0.004 and  $a$  the atomic weight.  $\alpha$  and  $\beta$  depend only on valence. But here, also, double bonds made their effect felt, though less pronouncedly than in refraction. Double bonded molecules have, in general, a lower molecular susceptibility than that calculated. Other effects of molecular form are seen; for example, the benzene nucleus augments the diamagnetism, although the double bonds it is supposed to contain should have an opposite effect. The factor of molecular structure appears, then, to influence diamagnetism. On the whole, however, calculation of the number of valences in the molecule by this procedure agrees better with the calculation from the cohesion, than does the calculation from the refractivity or dispersion. Thus, double bonds are of less action on the diamagnetism and in the benzene nucleus they

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<sup>1</sup> Pascal: "Sur un mode de contrôle optique des analyses magnétochimiques," *Comptes rendus*, **152**, 1852 (1911). *Recherches magnéto-chimiques sur la structure atomique des halogènes*, " *Comptes rendus*, **152**, 826 (1911). *Ann. Chim. Phys.*, [8] **19**, 1-80 (1910).



appear to exert no effect. By this method, as by the cohesion, all organic chlorine compounds examined were found to have trivalent chlorine and fluorine was monovalent. The agreement was good in regard to other elements also.

The connection between cohesion and diamagnetism is, therefore, again an indirect one. Both involve the molecular weight and the number of valences, but it is clear that cohesion is independent of molecular form, or very largely so; whereas whether a substance is magnetic, or diamagnetic, may depend, in part upon this very factor. Oxygen in an elemental form, is paramagnetic, not diamagnetic, and is quite anomalous in Pascal's scheme; whereas the cohesion of oxygen is not anomalous. In other words, whether a body is, as a whole, paramagnetic or diamagnetic, and to what degree, depends, probably, on the possibility of the orientation of the molecules, their polarity, etc., factors which do not seem to affect their cohesion or gravitation. Nevertheless cohesion and magnetic properties are, no doubt, closely related, since both depend on the same molecular properties, only magnetism involves still other properties, (form) not involved in cohesion.

The fact that cohesion is thus determined by the number of electron couples (atomic and valence) in the molecule plainly points toward the conclusion that cohesion is either electro-static or electro-magnetic in nature. Both of these possibilities have already been suggested. Sutherland,<sup>1</sup> from his discovery of the relation of cohesion to valence in salts, inferred at first that the cohesion must be of an electromagnetic nature. He supposed these rotating electron couples acted like little magnets, and he attempted to show, though whether successfully, or not, I am unable to judge, that small magnets, at sufficient distances apart, would attract inversely as the fourth power of the distance between them, and this he supposed to be the law of molecular attraction. This conclusion was attacked by van der Waals, Jr.,<sup>2</sup> who concluded, also

<sup>1</sup> Sutherland: *Phil. Mag.*, [6] 19, 1 (1910); 4, 625 (1902).

<sup>2</sup> Van der Waals, Jr.: *Kon. Akad. v. Wetensch. te Amsterdam, Proceedings*, 11, 132 (1908-1909).

from mathematical reasoning, that the attraction between such magnets would be inversely as the 7th or 9th power of the distance, and thus agree with the assumptions of his father, that molecular attraction diminished at such a rate that it was effective only when the molecules were in contact. Later, Sutherland<sup>1</sup> concluded that cohesion was due to electrostatic affinity of these electron couples. Lodge<sup>2</sup> made a similar suggestion. He thought some of the lines of force between the atoms wandered outside the molecule to atoms of other molecules and thus produced molecular cohesion. This would make molecular cohesion of the same nature as chemical affinity. While the relation between the two is close, both being zero in the absence of an electric charge, or valence, one is not causally dependent on the other, although both depend on the valences. The attraction between the atoms is probably of an electrostatic kind and, if so, should vary inversely as the square of the distance. The atomic weight does not appear to play a part in chemical affinity, for very light elements may enter into very firm union. In cohesion, molecular weight does play a large part; and while it is not impossible that the cohesion attraction may be inversely as the square of the distance, it is not probable, or at any rate it has not yet been proved to general satisfaction.

It seems much more probable to me that cohesion is more closely related to magnetism than to electrostatic affinity and I would raise the question whether magnetism is anything else than molecular cohesion made apparent at distances more than molecular. Is it not possible that molecular cohesion, involving as it does both atomic and valence electrons (atomic weight and valence) is due, perhaps, to the magnetic effects produced by the movements of these electron couples? In this view the atoms would be united by their electrostatic affinities and these same valences and the other atomic electrons by their magnetic effects produce the molecular

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<sup>1</sup> Sutherland: *Phil. Mag.*, [6] 17, 667 (1909).

<sup>2</sup> Lodge: *Nature*, 70, 176 (1904).



cohesion. I may state briefly some of the reasons which appear to lead to such a conclusion.

In the first place we have the surprising fact that the field of cohesion of a molecule is apparently delimited by the surrounding molecules. The evidence for this, while perhaps not conclusive, is both direct and indirect. The reason for the shortness of the radius of action of cohesion is one of the most interesting questions of molecular physics. It is of interest to see how this question came to be generally considered closed and settled in favor of the view, now generally accepted, that cohesive attraction diminishes with the distance at a rate far greater than gravitational attraction. It is chiefly due to Laplace.

Laplace,<sup>1</sup> in his beautiful memoir on capillarity, first raised the question whether the short radius of attraction of the cohesive forces was due to the fact that matter shut off the attraction, or was due to the attraction diminishing with the distance at a rate far more rapid than gravitation.

He says, when discussing Hawksbee's well-known experiments proving that the height to which water rises in a glass tube is independent of the thickness of the wall of the tube: "Hawksbee a observé que dans les tubes de verre, ou très minces ou très épais, l'eau s'élevait à la même hauteur toutes les fois que les diamètres intérieurs étaient les mêmes. Les couches cylindriques du verre qui sont à une distance sensible de la surface intérieure ne contribuent donc point à l'ascension de l'eau, quoique dans chacune d'elles, prise séparément, ce fluide doive s'élever au-dessus du niveau. Ce n'est point l'interposition des couches qu'elles embrassent qui arrête leur action sur l'eau, *car il est naturel de penser que les attractions capillaires se transmettent à travers les corps, ainsi que la pesanteur; cette action ne disparaît donc qu'à raison de la distance du fluide à ces couches, d'où il suit que l'attraction du verre sur l'eau n'est sensible qu'à des distances insensibles.*" I have italicized the end of Laplace's statement to bring out

<sup>1</sup> Laplace: "Sur l'action capillaire. Oeuvres. Supp. au Livre X," *Traité de Mécanique Céleste*, p. 351; see also p. 487.

clearly the reason which led him to the conclusion that molecular cohesion penetrated matter like gravitation, and that the attraction must, hence, decrease very rapidly with the distance. It will be seen that the sole reason for his decision was the possible analogy between gravitation and molecular cohesion.

The very important result of the rejection by Laplace of the possibility of cohesive attraction not penetrating matter was that it forced him to the conclusion that the cohesive force must diminish far more rapidly than gravitation as the distance increases. Laplace did not make any assumption as to the rate at which the cohesion attraction diminished with the distance, except that it was at so rapid a rate that the cohesion became negligible within all measurable distances.

The great English philosopher, Thomas Young,<sup>1</sup> who a year before Laplace had shown the true nature of surface tension and practically anticipated all of Laplace's main conclusions, does not appear to have raised the question in a concrete form. His papers on capillarity are so condensed that the reasoning is very difficult to follow.<sup>2</sup>

But while Young nowhere specifically puts the question whether the cohesion attraction penetrates matter, he made an assumption which might be taken to indicate that it does not. "We may suppose," he says (p. 43), "the particles of liquids, and probably those of solids also, to possess that power of repulsion which has been demonstratively shown by Newton to exist in aeriform fluids, and which varies as the simple inverse ratio of the distance of the particles from each other. In air and vapors this force appears to act

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<sup>1</sup> Young: "An Essay on the Cohesion of Fluids," *Phil. Trans.*, 1805 (collected works, edited by G. Peacock, 1, 418 (1855), London).

<sup>2</sup> A propos of this paper of Young's, Clerk Maxwell makes an interesting comment. He says: "His [Young's] essay contains the solution of a great number of cases including most of those afterwards solved by Laplace; but his methods of demonstration, though always correct and often extremely elegant, are sometimes rendered obscure by his scrupulous avoidance of mathematical symbols." *Ency. Brit.*, Article, "Capillarity."



uncontrolled; but in liquids it is overcome by a cohesive force, while the particles still retain a power of moving freely in all directions; and in solids the same cohesion is accompanied by a stronger or weaker resistance to all lateral motion, which is perfectly independent of the cohesive force and which must be cautiously distinguished from it." *"It is sufficient to suppose the force of cohesion nearly or perfectly constant in its magnitude throughout the minute distance to which it extends, and owing its apparent diversity to the contrary action of the repulsive force, which varies with the distance. Now, in the internal parts of a liquid, these forces hold each other in a perfect equilibrium, the particles being brought so near that the repulsion becomes precisely equal to the cohesive force that urges them together,"* etc.

Young thus assumed that the cohesion extended but a short distance, with slight variation in intensity and that it then ended abruptly. So far as I can find, he made no suggestion how it came to end abruptly; but if it be assumed that it does not penetrate matter, it is seen that it must end abruptly at the next layer of molecules. Young tried to estimate how far the cohesive force really extended, and found a value surprisingly near the order of magnitude of that now known to be the distance apart of the centers of two molecules. His reasoning on this point is extremely ingenious, and is of interest as the first estimate of molecular dimensions.

Lord Rayleigh<sup>1</sup> says anent this computation of Young's: "One of the most remarkable features of Young's treatise is his estimate of the range "*a*" of the attractive force on the basis of the relation  $T = \frac{1}{3}aK$ . Never once have I seen it alluded to, and it is, I believe, generally supposed that the first attempt of this kind is not more than twenty years old. It detracts nothing from the merit of this wonderful speculation that a more precise calculation does not verify the numerical coefficient in Young's equation. The point is

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<sup>1</sup> Rayleigh: "On the Theory of Surface Forces," *Phil. Mag.*, [5] 30, 285-298, 456-475 (1890). Collected papers, 3, 396.

that the range of the cohesive forces is necessarily of the order  $T/K$ ."  $T$  is the surface tension and  $K$  the internal pressure. Lord Rayleigh, in his revision of Maxwell's classical account of capillarity in the new edition of the *Encyclopaedia Britannica* and in his many splendid writings on this subject, does not seem to have considered this question. All other writers whom I have consulted seem to have followed Laplace's lead and assumed, without evidence, that cohesion does penetrate matter like gravitation.

Thus, Gauss,<sup>1</sup> who introduced clear ideas of surface energy and the potential energy of fluids, writes as follows in 1830: "The ordinary attraction which is proportional to the square of the distance, and which permits the representation of all motions in the heavens with such good agreement, can be used in the explanation neither of capillary phenomena nor of adhesion and cohesion; a correctly carried out computation shows 'dass eine nach diesem Gesetze wirkende Anziehung eines beliebigen Körpers der zur Ausführung von Experimenten geeignet ist, d. h., dessen Masse im Vergleich mit der der Erde vernachlässigt werden kann, auf einem beliebig gelegenen, sogar den Körper berührenden Punkt, im Vergleich mit der Schwere verschwinden muss. Wir schliessen hieraus dass jenes Anziehungsgesetz in den kleinsten Abständen mit der Wahrheit nicht mehr übereinstimmt, sondern dass es eine Modification erfordert.'" In other words, the particles of the body exert, besides the attractive force of gravitations, still another force which is noticeable only in the smallest distances. All appearances show uniformly that the second part of the attractive force (the molecular attraction) is not noticeable in the smallest measurable distances. On the other hand, in unmeasurably small distances it may greatly surpass the first, which is proportioned to the square of the distance.

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<sup>1</sup> Gauss: "Allgemeine Grundlagen einer Theorie der Gestalt von Flüssigkeiten," Ostwald's "Klassiker der exakten Wissenschaften," No. 135, p. 1. "Commentationes societatis Regiae Scientiarum Göttingensis Recentiores," vii, 1830.



It was necessary for him (p. 21), to make some assumptions regarding the cohesion attraction which he represented as " $f$ " ( $r$ ),  $r$  being the radius of molecular action, and he accordingly adopted Laplace's view that  $f$  ( $r$ ) decreases far more rapidly than  $1/r^2$ , which is the law of gravitational attraction (p. 22). He says, speaking of cohesion attraction, or  $f$  ( $r$ ): "Da dieser Ausdruck etwas unbestimmtes hat so lange wir nicht eine Einheit zu Grunde legen, wollen wir vor allem darauf aufmerksam machen, dass wir die anziehende Kraft  $f$  ( $r$ ), ausgedrückt als eine Function des Abstandes  $r$ , mit einer Masse multipliziert denken müssen, damit sie mit der Gravitation  $g$  in den Dimensionen übereinstimmt. Der Sinn unserer Voraussetzung ist dann der folgende: Bezeichnet  $M$  irgend eine Masse derart, wie sie uns in Experimenten vorkommt, nämlich eine, die im Vergleich mit der ganzen Erde als verschwindend angesehen werden kann, dann muss  $M f$  ( $r$ ) immer merklich sein im Vergleich mit der Schwere, so lange  $r$  einen unseren Messungen zugänglichen, wenn auch noch so kleinen Wert hat."

Van der Waals, in all his earlier writings, including his famous essay on the continuity of the gaseous and liquid states published in 1869, assumed with Laplace that molecular cohesion was appreciable only very close to the molecule; indeed, the radius of action was less than the mean distance apart of the molecular centers. I have not been able to find in his essays any specific discussion of the question whether cohesion attraction penetrates matter, although he does discuss the radius of attraction of a molecule.<sup>1</sup> His general assumption was that the cohesion diminished very rapidly as the molecules separated. In one brief communication to the Amsterdam Academy of Sciences (1893-94, pp. 20-21) he states that he had derived a potential function, that is, a function expressing the potential energy of attraction of two

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<sup>1</sup> Van der Waals: "Bijdrage tot de Kennis van de Wet der Overeenstemmende Toestanden," *Verhandelingen Konik. Akad. van Wetenschappen*, 21, 5 (1881).

molecules, of the form  $-f \frac{e^{-\frac{r}{\lambda}}}{r}$ , as the potential of two mass points; and he goes on to say that this formula was based on two assumptions, namely that the attraction of two molecules is inversely as the square of the distance, and second, that the universal medium absorbs the lines of force. He thus assumes an absorption by the ether of the attraction, rather than by a molecule at a distance " $r$ ." In his paper published in 1903, in which the real molecular volume, the value of " $b$ ," is no longer considered constant and in which he has revised his formula for the isotherm in so important a manner, he does not specifically reraise the question of the penetration of matter by the cohesive attraction.

In a succession of papers like those of van der Waals' which represent a progressive succession of ideas, mutually conflicting ideas may, not unnaturally, be found. Thus, in his paper on the thermodynamic potential and capillarity,<sup>1</sup> a limiting intermediate layer of rapidly changing density is supposed to exist between the saturated vapor and the liquid, and the existence of such a layer would seem to the writer to presuppose that the radius of attraction at least in this layer must be several molecular diameters.

On the other hand, the following statement<sup>2</sup> (p. 121) is not entirely reconcilable with this view, and would be so only if the layers of which he speaks are at least equal in thickness to the distance the cohesive force extends. He supposes the cohesive pressure to be exerted only by the surface layer, but he states that exactly the same formulas are obtained if the fluid be considered to be made up of a series of layers of *molecular dimensions*. "If we consider the gas in a cylindrical vessel of constant area and divided into horizontal layers, the lowest attracts the next higher," etc. The sum of all partial amounts of work will be the same as if one considered

<sup>1</sup> Van der Waals: "Theorie thermodynamique de la Capillarité," Archives Néerlandaises des Sciences exactes et naturelles, 28, 121 (1895).

<sup>2</sup> Van der Waals: "Die Continuität des gasförmigen u. flüssigen Zustandes," Leipzig, p. 126 (1899).



only the attraction of the upper layer and the distance as that through which this layer would have been moved. It seems to me that, for this reasoning to be correct, we must assume the layers at least as thick as the radius of action of each layer of molecules. If these layers are of molecular diameters, it would seem that the cohesive force does not extend farther than a molecular diameter. This is not apparently consistent with the assumption elsewhere made to explain the transitional layer between vapor and liquid.

Plateau also followed Laplace. Sutherland, in his many papers on molecular cohesion, assumes that the cohesional attraction is inversely as the fourth power of the distance, but he does not, so far as I can find, discuss the question of the penetration of matter by cohesional attraction. But it is impossible for cohesion to vary inversely as the fourth power, if cohesion penetrates matter.

Kleeman<sup>1</sup> supposes that the attraction must be inversely as the 5th or some higher power of the distance. He has pointed out that cohesion cannot possibly be assumed to vary like gravitation inversely with the square of the distance, because the cohesional attraction is so much greater than gravitation. But Mills weakened the force of this objection, which he had overlooked in his early papers, by making the additional postulate that cohesion does not penetrate matter. For if it be assumed that the cohesion does not penetrate matter then only the layer of superficial molecules of two masses would attract each other; and the number of these is so small, compared to the whole number of molecules in the mass, that the cohesional attraction would be less perceptible at sensible distances than gravitational attraction.

I have been unable, then, to find any evidence for the assumption so generally made that cohesive attraction penetrates matter like gravitation. There is no direct evidence, therefore, so far as I can find, against the inference necessitated

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<sup>1</sup> Kleeman: "An Investigation of the Determination of the Law of Chemical Attraction between Atoms from Physical Data," *Phil. Mag.*, [6] 21, 83 (1911).

by the square or fourth power law of attraction, that cohesion does not penetrate matter.

There is, on the other hand, some evidence of a direct kind that cohesion extends only as far as the nearest molecules. This evidence is the length of the radius of action as determined by direct measurement, and Einstein's proof that the radius of action varies with the distance apart of the molecular centers.

Laplace believed that the radius of action, although short, nevertheless extended many molecular diameters and this opinion prevailed until recently, but as means of measurement have improved the radius has shrunk. Quincke gave an estimate of about  $6 \times 10^{-6}$  cm, but the most recent determinations of Johannot,<sup>1</sup> and Chamberlain<sup>2</sup> show it to be about  $1.6-2 \times 10^{-7}$  cm in a soap film and in the case of glass. The diameter of a molecule of trioleate of glycerine, according to Perrin,<sup>3</sup> is  $1.1 \times 10^{-7}$  cm. The radius of action is certainly not more than two molecular diameters and indeed is hardly more than one. The average distance between the centers of two molecules of ether in the liquid state at  $20^{\circ}$  is about  $5.5 \times 10^{-8}$  cm.

But not only has direct measurement shown the radius of action to be one or two molecular diameters, but computations of it by Kleeman make it very close to this. For example, in ether Kleeman<sup>4</sup> computed the radius to be about  $3.4 \times 10^{-8}$  cm which is about the distance when the molecules are in contact. Van der Waals supposed it, indeed, to be only as long as this. Recently Einstein<sup>5</sup> has made a very interesting computation starting from the law of Eötvös, by which he shows, from thermodynamic reasoning, that the

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<sup>1</sup> Johannot: *Phil. Mag.*, [5] 47, 501 (1899).

<sup>2</sup> Chamberlain: *Phys. Rev.*, 31, 170 (1910).

<sup>3</sup> Devaux: *Journal de Physique*, [5] 2, 699 (1912).

<sup>4</sup> Kleeman: "On the Radius of the Sphere of Action," *Phil. Mag.*, [6] 19, 840 (1910).

<sup>5</sup> Einstein: "Bemerkung zu dem Gesetz von Eötvös," *Annalen der Physik.*, [4] 34, 165 (1911).



range of cohesive action must be of the general value of, and proportional to, the distance between the molecular centers. This distance is of the order of magnitude in most liquids of  $10^{-8}$  cm. This result is so surprising that Einstein says of it: "This result appears at first very unlikely, for what should the radius of action of a molecule have to do with the distance between neighboring molecules? The supposition is only reasonable in case the neighboring molecules alone attract each other, but not those farther removed."

Sutherland<sup>1</sup> also came to the conclusion that the radius of action was about equal to  $v^{1/3}$ . We see then, that the evidence points to the conclusion that the radius of action of the molecular forces agrees very closely with  $v^{1/3}$ , the distance between the molecular centers, and varies directly with  $v^{1/3}$ . The only explanation of this fact appears to me to be that the attraction does not extend beyond neighboring molecules and, hence, must, in some way, be stopped by them.

Mills<sup>2</sup> alone, so far as I can find, has reopened the question whether the field is delimited by the surrounding molecules. Concluding, I believe erroneously, from an empirical law, that the attraction between molecules must vary inversely as the square of the distance, he was driven to the second conclusion that if this were the case the cohesion could not penetrate matter. He assumed, hence, that the surrounding molecules absorbed, or neutralized, the lines of cohesive force. The direct evidence and Einstein's reasoning leaves little doubt that the radius varies with the distance apart of the molecules and the only possible conclusion from this is that the surrounding molecules delimit the field as Mills supposes.

If it is a fact that the surrounding molecules delimit the field as the evidence indicates, cohesion is allied at once with magnetism, for this is the very supposition which Ewing made to explain some of the phenomena of magnetism. Each molecule of a ferromagnetic substance is supposed to be a

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<sup>1</sup> Sutherland: *Phil. Mag.*, [6] 4, 632, 636 (1902).

<sup>2</sup> Mills: *Jour. Phys. Chem.*, 15, 417 (1911).

magnet. In the non-magnetic state the magnetism of each molecule is supposed to be neutralized by the surrounding molecules, which have their magnetic axes variously directed. The magnetic field is thus limited to a single molecular diameter and will vary with the distance apart of the molecules. The magnetic field of each molecule is delimited by the surrounding molecules. If, however, these molecules are oriented, either by acting on each other, or by external forces, then the magnetic fields coincide and the magnetism may be perceived extending outward from the mass. In other words, to explain why magnetism does not persist in soft iron and to account for magnetic hysteresis, Ewing has made exactly the same assumption which has been made to explain why cohesion does not extend beyond molecular distances.

It seems to me not impossible that magnetism is the cohesive attraction of a molecule. If the molecule is of such a nature, or shape, that the total effect of the little magnets, its electron couples, coincide more or less completely so that the molecule has a polarity, and the molecules can be oriented in any way and held in position, we have the ferro and paramagnetic substances. If the molecules have many poles, so that there is no polarity of the molecules as a whole, there is a diamagnetic substance. The magnetic field of each molecule would be its cohesive field.

The recent work of Cotton and Mouton<sup>1</sup> on the orientation of molecules in the magnetic field seems to me to bear out such an interpretation. The work of Weiss<sup>2</sup> and Langevin<sup>3</sup> appears to point in the same direction, but Weiss, who has considered the possibility of the identity of cohesion and magnetism, states that he will shortly show that they can not be identical. Nevertheless it appears to me not impossible that magnetism is simply a special case of cohesion, and if this is true the rotation of the plane of polarized light by optically active substances would be easily understood.

<sup>1</sup> Cotton and Mouton: *Journal de Physique*, [5] 1, 40 (1911).

<sup>2</sup> Weiss: *Journal de Physique*, [5] 1, 900 (1911); [4] 6, 661 (1907).

<sup>3</sup> Langevin: *Ann. Chim. Phys.*, 5, 70 (1905).



Finally, if it is true that the surrounding molecules delimit the field of cohesion in any way whatever, and that only six molecules really take part in this delimitation, we can derive the value  $a/V^2$  of van der Waals' at once and very simply.

Suppose that each molecule has a certain mass of cohesion,  $M$ , and that two molecules attract each other directly as the product of their cohesive masses and inversely as the fourth power of the distance between their centers, then the attraction between two molecules would be  $M^2K/v^{4/3}$ , where  $v$  is the space at the disposal of a single molecule. Since each molecule attracts only the one above, below and to each side, the pressure per square centimeter of a double layer of molecules will be  $M^2K/v^{4/3}$  multiplied by the number of molecules in 1 sq. cm or  $1/v^{2/3}$ , making  $M^2K/v^2$ . Since the attraction extends only a single molecular diameter, we may multiply both numerator and denominator by  $N^2$ , where  $N$  is the number of molecules in the volume,  $V$ , and we obtain,  $N^2M^2K/N^2v^2 = M^2N^2K/V^2 = a/V^2$ .  $M^2K$  has already been shown to be  $2.98 \times 10^{-37}$  (Mol. Wt.  $\times$  Valences)<sup>2/3</sup>.

We have, as yet, no proof that only the six surrounding molecules are attracted, although Sutherland<sup>1</sup> has made a similar supposition. But such may be the case nevertheless. Einstein, in his calculation, computed that each molecule could be considered as lying at the center of a cube and that it attracted the 26 other molecules of the cube. Of course the fact that the value  $a/V^2$  may be so easily derived in this way does not furnish any proof that the attraction is inversely as the fourth power of the distance. But I know of no other derivation of  $a/V^2$  which involves so few, or less radical, assumptions.

The general conclusion of the paper is then, that cohesion,

<sup>1</sup> Sutherland: Phil. Mag., [6] 17, 667 (1909). "The total potential energy of a number of like molecules is the same as if each caused its own domain to be uniformly electrized with an electric moment proportional to the linear dimensions of the domain, the direction of electrization being such that in general any molecule attracts its six immediate neighbors."

being a function of molecular weight and valence, is a function of the number of electron couples of the valences and atoms, and is, hence, probably of a magnetic nature. Magnetic substances may be supposed to be substances in which, owing to the orientation of the molecules, or to their polarity, or both these causes, the cohesive fields of the molecules are not delimited or neutralized by the surrounding molecules so that the cohesion attraction becomes apparent at more than molecular distances, and under such circumstances the substance is said to be magnetic.

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# THE SIGNIFICANCE OF THE RELATIONSHIP BETWEEN MOLECULAR COHESION AND THE PRODUCT OF THE MOLECULAR WEIGHT AND THE NUMBER OF VALENCES

BY ALBERT P. MATHEWS

In the preceding papers<sup>1</sup> of this series I have shown that the value of "*a*" of van der Waals, representing molecular cohesion, or the value  $M^2K$  which is the factor "*a*" for a single molecule, is proportional to the two-thirds power of the product of the molecular weight by the number of valences of the molecule.  $M^2K$  was found to be equal, when expressed in absolute units, to  $2.98 \times 10^{-37}$  (Mol. Wt.  $\times$  No. of Val.)<sup>2/3</sup>.

In this paper I shall discuss the theoretical bearing of the relationship of cohesion to these molecular properties.

Attempts have been made by others to correlate cohesion, or "*a*," with molecular weight and the number of valences, but with very partial success. Sutherland<sup>2</sup> at first supposed the molecular attraction to be proportional to the product of the gravitational masses of the molecules. This he found would not do, and in his later papers he stated that the gravitational mass of a molecule did not enter into the expression "*a*." Amagat,<sup>3</sup> also, recently revived the idea that gravitational mass plays a role in cohesion and suggested that  $a/V^2$  ought to be proportional to the square of the molecular mass. This, however, he did not find to be the case. Leduc<sup>4</sup> has recently confirmed, in part, this view of Amagat's for gases of similar molecular composition, when taken under the same volume and at corresponding temperatures. Kleeman,<sup>5</sup> also has tried to find a relationship between "*a*" and gravita-

<sup>1</sup> Mathews: Jour. Phys. Chem., 17, 154 (1913).

<sup>2</sup> Sutherland: Phil. Mag., [5] 27, 305 (1889); [6] 4, 632 (1902).

<sup>3</sup> Amagat: "Pression interne des fluides," Journal de Physique, [4] 8, 617 (1909).

<sup>4</sup> Leduc: Comptes rendus, 153, 179 (1911).

<sup>5</sup> Kleeman: Phil. Mag., [6] 19, 783, 840-847 (1910).

tional mass, and states that the cohesive attraction of two molecules is proportional to the product of the two sums of the square roots of the atomic weights of the atoms of the molecules. This relationship, however, is of very limited applicability, if indeed, it correctly expresses the cohesion of any.

As regards valence, I can find but one other suggestion, that of Sutherland.<sup>1</sup> He showed that the number of equivalents, or valences, in simple substances, such as sodium chloride, influenced the value of their cohesion. He was unable to establish this relationship for more complex bodies. Nevertheless he assumed that it existed in them and correctly surmised from it the relationship between cohesion and chemical affinity, and adduced it as evidence of the electrostatic or magnetic nature of cohesion. "*a*" was made proportional to the square root of the valence.

The relationship between cohesion and the properties of molecular weight and the number of valences can be interpreted best by Sir J. J. Thomson's theory of the electrical constitution of matter and valence, and, so far as I can see, on no other hypothesis. It speaks, therefore, for the electrostatic, or electro-magnetic theory of cohesion, and, in my opinion, for the latter.

The relation,  $M^2K = (f) \text{Val}^{2/3} / \text{Mol. Wt.}^{2/3}$ , seems at first peculiar. It is odd that the valence of an atom should be of as much importance in cohesion as the weight of the atom; it is a relationship which one would not have anticipated. The significance of this fact, if I am not mistaken, is that the electron couples constituting the molecules are of two kinds, namely, those of the atoms themselves, which added together presumably give the molecular weight; and the valence electrons, which differ from the others so that they cannot be added to them. Hence the formula is not  $M^2K = (f) (\text{Wt.} + \text{Val.})$ , the cohesion being proportional to the sum; but the mass of cohesion is proportional to the cube root of each of

<sup>1</sup> Sutherland: *Phil. Mag.*, [6] 4, 632 (1902).



these kinds of electrons and so is proportional to the cube root of their product. The valence electrons are probably more labile, more easily removed and replaced. They have a different degree of liberty and they cannot be summed with the atomic.

The formula thus confirms the correctness of Drude's promise that the electrons of the valences differ in their properties from the electrons of the atoms. He concluded that only the valence electrons would be sufficiently free to vibrate synchronously with light and hence these electrons must be particularly concerned in the refraction and dispersion of light. Drude's<sup>1</sup> suggestion of electrons of different degrees of liberty confirmed, as it was, by experiments showing a relation between valence and dispersion, is thus confirmed also from the wholly different field of cohesion.

A still more interesting conclusion may be drawn from this relationship, namely, that a neutral, uncharged atom having no valence will have no cohesion. Since it will have no chemical affinity either, if chemical affinity is, as it appears to be, of an electrical nature, it is thus seen that a close relation must exist between chemical affinity and cohesion. Such neutral atoms will presumably still have gravitational attraction. A free electrical charge on the atom is, therefore, necessary for cohesion, but not for gravitation. Furthermore, the cohesive effect is the same whether the charge be positive or negative; and it is proportional to the number of charges. The formula shows, also, that the effect of a free charge on any atom is proportional to the weight of the atom; that is, the effect of the valence charge is multiplied, as it were, by the number of electron couples in the atom; and the effect of the total number of valence charges in the molecule is multiplied by the whole number of atomic electron couples in the molecule. Just how such an effect could be produced, and why the attraction, or cohesive mass, should ultimately prove to be proportional to a linear function (the cube root) of the product

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<sup>1</sup> Drude: *Annalen der Physik.*, [4] 14, 677 (1904).

of the number of valences by the molecular weight, I do not see.

It appears, then, that refraction, dispersion and cohesion all involve the valence electrons, but the connection between cohesion and valence is far closer and simpler than the other relationships appear to be. The relationship of valence to light is necessarily a less direct one, refraction depending on the rate of vibration of the electron. It is said<sup>1</sup> that if the natural period of the molecule (electron) is slightly less than the frequency of a light wave the light will be accelerated; if greater, retarded. It is evident that in dispersion other properties of the electrons than number come into play, and, hence, the relationship between dispersion and number is not so simple and direct. Double bonds, neighboring groups, etc., influence the periods of the electrons and so influence the dispersive power; whereas these factors appear to play no important part in cohesion.

The relation between the refraction of light of one wave length and the valence number is still less direct than between dispersion and valence, but still a general relation exists which for substances of the same type is rather uniform, as shown by Traube<sup>2</sup> for many liquids and by Cuthbertson<sup>3</sup> for several gases.

Another very interesting fact correlating the refractive and cohesive properties of matter is the resemblance between the constant "K" of the Ketteler dispersion formula and the value  $M^2K$  of cohesion. Thus with the Ketteler formula  $n^2 = a^2 - K\lambda^2 + D\lambda^2_v/(\lambda^2 - \lambda^2_v)$  the constant "K," Drude found, could be computed with a fair approximation, in some cases at any rate, from the sum of the valences, the molecular weight and the density, and this result was confirmed by Erfle.<sup>4</sup> This constant "K," therefore, contains at least

<sup>1</sup> Cotter, J. R.: "Dispersion," *Encyclo. Brit.*, 11th edition, 8, 317.

<sup>2</sup> Traube: *Ber. chem. Ges. Berlin*, 40, 130 (1907).

<sup>3</sup> Cuthbertson: *Proc. Roy. Soc.*, 83A (1909-1910); *Phil. Mag.*, [6] 21, 69 (1911); *Phil. Trans.*, 204, 323 (1905); 207, 135 (1907).

<sup>4</sup> Erfle: "Optische Eigenschaften und Elektronen Theorie." *Annalen der Physik*, [4] 24 (1907).



sometimes the same factors as the value  $a/V^2$  of van der Waals' equation. I have not, however, attempted to establish any closer connection between them.<sup>1</sup>

We conclude, therefore, that while cohesion and refractivity are both dependent on a common factor, namely the valence electrons, and possibly upon the molecular weight, the connection between them is not direct, but indirect; and while cohesion and refraction, or dispersion, often parallel each other, they, at other times, diverge considerably since other factors enter into refraction.

It is not without interest to recall as an example of the perspicacity of genius, that Laplace<sup>2</sup> long ago foretold a connection between these properties. Writing in 1805 of the formula of capillarity which, as will be remembered, contained two terms, one  $K$ , representing molecular cohesion, or van der Waals' expression  $a/v^2$ ; the other,  $H$ , the capillary constant, Laplace says (p. 351): "I saw that this action (pressure) is smaller or larger than if the surface is plane; smaller, if the surface is concave; larger, if it is convex. Its analytical expression is composed of two terms: the first ( $K$ ), much larger than the second, expresses the action of the mass terminated by a plane surface; and I think from this term depends the suspension of mercury in a barometer tube at a height two to three times greater than that due to atmospheric pressure, the refractive powers of diaphanous bodies, the cohesion, and in general, chemical affinity; the second term expresses the part of the action due to the sphericity of the surface." And again (p. 362): "The function,  $K$ , is analogous to that I have designated by the same letter in the refraction of light."

But of even greater interest and more fundamental importance than the relation between the optical and the cohesive properties, which is now understandable since both

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<sup>1</sup> See also Natanson: *Bull. de l'Acad. des Sci. de Cracovie*, 1907, April p. 316 for the relation of refraction and valence.

<sup>2</sup> Laplace: *Sur l'action capillaire. Oeuvres. Supp. Liv. X, Traité de Mécanique Céleste*, p. 351.

involve the number of valence electrons, is the relation between the magnetic and cohesive properties, since here we touch, I think, the very kernel of the problem of the nature of cohesion.

The connection between the magnetic properties and cohesion is brought out very clearly, in an empirical way, by Pascal's<sup>1</sup> investigations on the relation between magnetic susceptibility and the molecular properties. In a series of papers Pascal has shown that there is a remarkable connection between the specific susceptibility of diamagnetic elements and the atomic weights and valences. Thus if elements of the same family having the same valence are arranged in their order of increasing specific susceptibility, they are in the order of their atomic weights; and, on the other hand, a close dependence on valence may be observed. In elements of nearly the same weight but of different valence numbers, the atomic magnetic susceptibility,  $X_a$ , increases with the number of valences. An empirical relationship was found between them,  $X_a = -10^{-7}e^{\alpha + \beta a}$  where  $a$  is about 2.1,  $\beta$  about 0.004 and  $a$  the atomic weight.  $\alpha$  and  $\beta$  depend only on valence. But here, also, double bonds made their effect felt, though less pronouncedly than in refraction. Double bonded molecules have, in general, a lower molecular susceptibility than that calculated. Other effects of molecular form are seen; for example, the benzene nucleus augments the diamagnetism, although the double bonds it is supposed to contain should have an opposite effect. The factor of molecular structure appears, then, to influence diamagnetism. On the whole, however, calculation of the number of valences in the molecule by this procedure agrees better with the calculation from the cohesion, than does the calculation from the refractivity or dispersion. Thus, double bonds are of less action on the diamagnetism and in the benzene nucleus they

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<sup>1</sup> Pascal: "Sur un mode de contrôle optique des analyses magnétochimiques," *Comptes rendus*, **152**, 1852 (1911). *Recherches magnéto-chimiques sur la structure atomique des halogènes*, " *Comptes rendus*, **152**, 826 (1911). *Ann. Chim. Phys.*, [8] **19**, 1-80 (1910).



appear to exert no effect. By this method, as by the cohesion, all organic chlorine compounds examined were found to have trivalent chlorine and fluorine was monovalent. The agreement was good in regard to other elements also.

The connection between cohesion and diamagnetism is, therefore, again an indirect one. Both involve the molecular weight and the number of valences, but it is clear that cohesion is independent of molecular form, or very largely so; whereas whether a substance is magnetic, or diamagnetic, may depend, in part upon this very factor. Oxygen in an elemental form, is paramagnetic, not diamagnetic, and is quite anomalous in Pascal's scheme; whereas the cohesion of oxygen is not anomalous. In other words, whether a body is, as a whole, paramagnetic or diamagnetic, and to what degree, depends, probably, on the possibility of the orientation of the molecules, their polarity, etc., factors which do not seem to affect their cohesion or gravitation. Nevertheless cohesion and magnetic properties are, no doubt, closely related, since both depend on the same molecular properties, only magnetism involves still other properties, (form) not involved in cohesion.

The fact that cohesion is thus determined by the number of electron couples (atomic and valence) in the molecule plainly points toward the conclusion that cohesion is either electro-static or electro-magnetic in nature. Both of these possibilities have already been suggested. Sutherland,<sup>1</sup> from his discovery of the relation of cohesion to valence in salts, inferred at first that the cohesion must be of an electromagnetic nature. He supposed these rotating electron couples acted like little magnets, and he attempted to show, though whether successfully, or not, I am unable to judge, that small magnets, at sufficient distances apart, would attract inversely as the fourth power of the distance between them, and this he supposed to be the law of molecular attraction. This conclusion was attacked by van der Waals, Jr.,<sup>2</sup> who concluded, also

<sup>1</sup> Sutherland: *Phil. Mag.*, [6] 19, 1 (1910); 4, 625 (1902).

<sup>2</sup> Van der Waals, Jr.: *Kon. Akad. v. Wetensch. te Amsterdam, Proceedings*, 11, 132 (1908-1909).

from mathematical reasoning, that the attraction between such magnets would be inversely as the 7th or 9th power of the distance, and thus agree with the assumptions of his father, that molecular attraction diminished at such a rate that it was effective only when the molecules were in contact. Later, Sutherland<sup>1</sup> concluded that cohesional attraction was due to electrostatic affinity of these electron couples. Lodge<sup>2</sup> made a similar suggestion. He thought some of the lines of force between the atoms wandered outside the molecule to atoms of other molecules and thus produced molecular cohesion. This would make molecular cohesion of the same nature as chemical affinity. While the relation between the two is close, both being zero in the absence of an electric charge, or valence, one is not causally dependent on the other, although both depend on the valences. The attraction between the atoms is probably of an electrostatic kind and, if so, should vary inversely as the square of the distance. The atomic weight does not appear to play a part in chemical affinity, for very light elements may enter into very firm union. In cohesion, molecular weight does play a large part; and while it is not impossible that the cohesional attraction may be inversely as the square of the distance, it is not probable, or at any rate it has not yet been proved to general satisfaction.

It seems much more probable to me that cohesion is more closely related to magnetism than to electrostatic affinity and I would raise the question whether magnetism is anything else than molecular cohesion made apparent at distances more than molecular. Is it not possible that molecular cohesion, involving as it does both atomic and valence electrons (atomic weight and valence) is due, perhaps, to the magnetic effects produced by the movements of these electron couples? In this view the atoms would be united by their electrostatic affinities and these same valences and the other atomic electrons by their magnetic effects produce the molecular

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<sup>1</sup> Sutherland: *Phil. Mag.*, [6] 17, 667 (1909).

<sup>2</sup> Lodge: *Nature*, 70, 176 (1904).



cohesion. I may state briefly some of the reasons which appear to lead to such a conclusion.

In the first place we have the surprising fact that the field of cohesion of a molecule is apparently delimited by the surrounding molecules. The evidence for this, while perhaps not conclusive, is both direct and indirect. The reason for the shortness of the radius of action of cohesion is one of the most interesting questions of molecular physics. It is of interest to see how this question came to be generally considered closed and settled in favor of the view, now generally accepted, that cohesive attraction diminishes with the distance at a rate far greater than gravitational attraction. It is chiefly due to Laplace.

Laplace,<sup>1</sup> in his beautiful memoir on capillarity, first raised the question whether the short radius of attraction of the cohesive forces was due to the fact that matter shut off the attraction, or was due to the attraction diminishing with the distance at a rate far more rapid than gravitation.

He says, when discussing Hawksbee's well-known experiments proving that the height to which water rises in a glass tube is independent of the thickness of the wall of the tube: "Hawksbee a observé que dans les tubes de verre, ou très minces ou très épais, l'eau s'élevait à la même hauteur toutes les fois que les diamètres intérieurs étaient les mêmes. Les couches cylindriques du verre qui sont à une distance sensible de la surface intérieure ne contribuent donc point à l'ascension de l'eau, quoique dans chacune d'elles, prise séparément, ce fluide doive s'élever au-dessus du niveau. Ce n'est point l'interposition des couches qu'elles embrassent qui arrête leur action sur l'eau, car il est naturel de penser que les attractions capillaires se transmettent à travers les corps, ainsi que la pesanteur; cette action ne disparaît donc qu'à raison de la distance du fluide à ces couches, d'où il suit que l'attraction du verre sur l'eau n'est sensible qu'à des distances insensibles." I have italicized the end of Laplace's statement to bring out

<sup>1</sup> Laplace: "Sur l'action capillaire. Oeuvres. Supp. au Livre X," *Traité de Mécanique Céleste*, p. 351; see also p. 487.

clearly the reason which led him to the conclusion that molecular cohesion penetrated matter like gravitation, and that the attraction must, hence, decrease very rapidly with the distance. It will be seen that the sole reason for his decision was the possible analogy between gravitation and molecular cohesion.

The very important result of the rejection by Laplace of the possibility of cohesive attraction not penetrating matter was that it forced him to the conclusion that the cohesive force must diminish far more rapidly than gravitation as the distance increases. Laplace did not make any assumption as to the rate at which the cohesional attraction diminished with the distance, except that it was at so rapid a rate that the cohesion became negligible within all measurable distances.

The great English philosopher, Thomas Young,<sup>1</sup> who a year before Laplace had shown the true nature of surface tension and practically anticipated all of Laplace's main conclusions, does not appear to have raised the question in a concrete form. His papers on capillarity are so condensed that the reasoning is very difficult to follow.<sup>2</sup>

But while Young nowhere specifically puts the question whether the cohesional attraction penetrates matter, he made an assumption which might be taken to indicate that it does not. "We may suppose," he says (p. 43), "the particles of liquids, and probably those of solids also, to possess that power of repulsion which has been demonstratively shown by Newton to exist in aeriform fluids, and which varies as the simple inverse ratio of the distance of the particles from each other. In air and vapors this force appears to act

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<sup>1</sup> Young: "An Essay on the Cohesion of Fluids," *Phil. Trans.*, 1805 (collected works, edited by G. Peacock, 1, 418 (1855), London).

<sup>2</sup> A propos of this paper of Young's, Clerk Maxwell makes an interesting comment. He says: "His [Young's] essay contains the solution of a great number of cases including most of those afterwards solved by Laplace; but his methods of demonstration, though always correct and often extremely elegant, are sometimes rendered obscure by his scrupulous avoidance of mathematical symbols." *Ency. Brit.*, Article, "Capillarity."



uncontrolled; but in liquids it is overcome by a cohesive force, while the particles still retain a power of moving freely in all directions; and in solids the same cohesion is accompanied by a stronger or weaker resistance to all lateral motion, which is perfectly independent of the cohesive force and which must be cautiously distinguished from it." *"It is sufficient to suppose the force of cohesion nearly or perfectly constant in its magnitude throughout the minute distance to which it extends, and owing its apparent diversity to the contrary action of the repulsive force, which varies with the distance. Now, in the internal parts of a liquid, these forces hold each other in a perfect equilibrium, the particles being brought so near that the repulsion becomes precisely equal to the cohesive force that urges them together,"* etc.

Young thus assumed that the cohesion extended but a short distance, with slight variation in intensity and that it then ended abruptly. So far as I can find, he made no suggestion how it came to end abruptly; but if it be assumed that it does not penetrate matter, it is seen that it must end abruptly at the next layer of molecules. Young tried to estimate how far the cohesive force really extended, and found a value surprisingly near the order of magnitude of that now known to be the distance apart of the centers of two molecules. His reasoning on this point is extremely ingenious, and is of interest as the first estimate of molecular dimensions.

Lord Rayleigh<sup>1</sup> says anent this computation of Young's: "One of the most remarkable features of Young's treatise is his estimate of the range "*a*" of the attractive force on the basis of the relation  $T = \frac{1}{3}aK$ . Never once have I seen it alluded to, and it is, I believe, generally supposed that the first attempt of this kind is not more than twenty years old. It detracts nothing from the merit of this wonderful speculation that a more precise calculation does not verify the numerical coefficient in Young's equation. The point is

<sup>1</sup> Rayleigh: "On the Theory of Surface Forces," *Phil. Mag.*, [5] 30, 285-298, 456-475 (1890). Collected papers, 3, 396.

that the range of the cohesive forces is necessarily of the order  $T/K$ ."  $T$  is the surface tension and  $K$  the internal pressure. Lord Rayleigh, in his revision of Maxwell's classical account of capillarity in the new edition of the *Encyclopaedia Britannica* and in his many splendid writings on this subject, does not seem to have considered this question. All other writers whom I have consulted seem to have followed Laplace's lead and assumed, without evidence, that cohesion does penetrate matter like gravitation.

Thus, Gauss,<sup>1</sup> who introduced clear ideas of surface energy and the potential energy of fluids, writes as follows in 1830: "The ordinary attraction which is proportional to the square of the distance, and which permits the representation of all motions in the heavens with such good agreement, can be used in the explanation neither of capillary phenomena nor of adhesion and cohesion; a correctly carried out computation shows 'dass eine nach diesem Gesetze wirkende Anziehung eines beliebigen Körpers der zur Ausführung von Experimenten geeignet ist, d. h., dessen Masse im Vergleich mit der der Erde vernachlässigt werden kann, auf einem beliebig gelegenen, sogar den Körper berührenden Punkt, im Vergleich mit der Schwere verschwinden muss. Wir schliessen hieraus dass jenes Anziehungsgesetz in den kleinsten Abständen mit der Wahrheit nicht mehr übereinstimmt, sondern dass es eine Modification erfordert.'" In other words, the particles of the body exert, besides the attractive force of gravitations, still another force which is noticeable only in the smallest distances. All appearances show uniformly that the second part of the attractive force (the molecular attraction) is not noticeable in the smallest measurable distances. On the other hand, in unmeasurably small distances it may greatly surpass the first, which is proportioned to the square of the distance.

<sup>1</sup> Gauss: "Allgemeine Grundlagen einer Theorie der Gestalt von Flüssigkeiten," Ostwald's "Klassiker der exakten Wissenschaften," No. 135, p. 1. "Commentationes societatis Regiae Scientiarum Göttingensis Recentiores," vii, 1830.



It was necessary for him (p. 21), to make some assumptions regarding the cohesional attraction which he represented as " $f$ " ( $r$ ),  $r$  being the radius of molecular action, and he accordingly adopted Laplace's view that  $f$  ( $r$ ) decreases far more rapidly than  $1/r^2$ , which is the law of gravitational attraction (p. 22). He says, speaking of cohesional attraction, or  $f$  ( $r$ ): "Da dieser Ausdruck etwas unbestimmtes hat so lange wir nicht eine Einheit zu Grunde legen, wollen wir vor allem darauf aufmerksam machen, dass wir die anziehende Kraft  $f$  ( $r$ ), ausgedrückt als eine Function des Abstandes  $r$ , mit einer Masse multipliziert denken müssen, damit sie mit der Gravitation  $g$  in den Dimensionen übereinstimmt. Der Sinn unserer Voraussetzung ist dann der folgende: Bezeichnet  $M$  irgend eine Masse derart, wie sie uns in Experimenten vorkommt, nämlich eine, die im Vergleich mit der ganzen Erde als verschwindend angesehen werden kann, dann muss  $M f$  ( $r$ ) immer merklich sein im Vergleich mit der Schwere, so lange  $r$  einen unseren Messungen zugänglichen, wenn auch noch so kleinen Wert hat."

Van der Waals, in all his earlier writings, including his famous essay on the continuity of the gaseous and liquid states published in 1869, assumed with Laplace that molecular cohesion was appreciable only very close to the molecule; indeed, the radius of action was less than the mean distance apart of the molecular centers. I have not been able to find in his essays any specific discussion of the question whether cohesional attraction penetrates matter, although he does discuss the radius of attraction of a molecule.<sup>1</sup> His general assumption was that the cohesion diminished very rapidly as the molecules separated. In one brief communication to the Amsterdam Academy of Sciences (1893-94, pp. 20-21) he states that he had derived a potential function, that is, a function expressing the potential energy of attraction of two

<sup>1</sup> Van der Waals: "Bijdrage tot de Kennis van de Wet der Overeenstemmende Toestanden," *Verhandelingen Konink. Akad. van Wetenschappen*, 21, 5 (1881).

molecules, of the form  $-f \frac{e^{-\frac{r}{\lambda}}}{r}$ , as the potential of two mass points; and he goes on to say that this formula was based on two assumptions, namely that the attraction of two molecules is inversely as the square of the distance, and second, that the universal medium absorbs the lines of force. He thus assumes an absorption by the ether of the attraction, rather than by a molecule at a distance " $r$ ." In his paper published in 1903, in which the real molecular volume, the value of " $b$ ," is no longer considered constant and in which he has revised his formula for the isotherm in so important a manner, he does not specifically reraise the question of the penetration of matter by the cohesive attraction.

In a succession of papers like those of van der Waals' which represent a progressive succession of ideas, mutually conflicting ideas may, not unnaturally, be found. Thus, in his paper on the thermodynamic potential and capillarity,<sup>1</sup> a limiting intermediate layer of rapidly changing density is supposed to exist between the saturated vapor and the liquid, and the existence of such a layer would seem to the writer to presuppose that the radius of attraction at least in this layer must be several molecular diameters.

On the other hand, the following statement<sup>2</sup> (p. 121) is not entirely reconcilable with this view, and would be so only if the layers of which he speaks are at least equal in thickness to the distance the cohesive force extends. He supposes the cohesive pressure to be exerted only by the surface layer, but he states that exactly the same formulas are obtained if the fluid be considered to be made up of a series of layers of *molecular dimensions*. "If we consider the gas in a cylindrical vessel of constant area and divided into horizontal layers, the lowest attracts the next higher," etc. The sum of all partial amounts of work will be the same as if one considered

<sup>1</sup> Van der Waals: "Theorie thermodynamique de la Capillarité," Archives Néerlandaises des Sciences exactes et naturelles, 28, 121 (1895).

<sup>2</sup> Van der Waals: "Die Continuität des gasförmigen u. flüssigen Zustandes," Leipzig, p. 126 (1899).



only the attraction of the upper layer and the distance as that through which this layer would have been moved. It seems to me that, for this reasoning to be correct, we must assume the layers at least as thick as the radius of action of each layer of molecules. If these layers are of molecular diameters, it would seem that the cohesive force does not extend farther than a molecular diameter. This is not apparently consistent with the assumption elsewhere made to explain the transitional layer between vapor and liquid.

Plateau also followed Laplace. Sutherland, in his many papers on molecular cohesion, assumes that the cohesive attraction is inversely as the fourth power of the distance, but he does not, so far as I can find, discuss the question of the penetration of matter by cohesive attraction. But it is impossible for cohesion to vary inversely as the fourth power, if cohesion penetrates matter.

Kleeman<sup>1</sup> supposes that the attraction must be inversely as the 5th or some higher power of the distance. He has pointed out that cohesion cannot possibly be assumed to vary like gravitation inversely with the square of the distance, because the cohesive attraction is so much greater than gravitation. But Mills weakened the force of this objection, which he had overlooked in his early papers, by making the additional postulate that cohesion does not penetrate matter. For if it be assumed that the cohesion does not penetrate matter then only the layer of superficial molecules of two masses would attract each other; and the number of these is so small, compared to the whole number of molecules in the mass, that the cohesive attraction would be less perceptible at sensible distances than gravitational attraction.

I have been unable, then, to find any evidence for the assumption so generally made that cohesive attraction penetrates matter like gravitation. There is no direct evidence, therefore, so far as I can find, against the inference necessitated

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<sup>1</sup> Kleeman: "An Investigation of the Determination of the Law of Chemical Attraction between Atoms from Physical Data," *Phil. Mag.*, [6] 21, 83 (1911).

by the square or fourth power law of attraction, that cohesion does not penetrate matter.

There is, on the other hand, some evidence of a direct kind that cohesion extends only as far as the nearest molecules. This evidence is the length of the radius of action as determined by direct measurement, and Einstein's proof that the radius of action varies with the distance apart of the molecular centers.

Laplace believed that the radius of action, although short, nevertheless extended many molecular diameters and this opinion prevailed until recently, but as means of measurement have improved the radius has shrunk. Quincke gave an estimate of about  $6 \times 10^{-6}$  cm, but the most recent determinations of Johannot,<sup>1</sup> and Chamberlain<sup>2</sup> show it to be about  $1.6-2 \times 10^{-7}$  cm in a soap film and in the case of glass. The diameter of a molecule of trioleate of glycerine, according to Perrin,<sup>3</sup> is  $1.1 \times 10^{-7}$  cm. The radius of action is certainly not more than two molecular diameters and indeed is hardly more than one. The average distance between the centers of two molecules of ether in the liquid state at  $20^{\circ}$  is about  $5.5 \times 10^{-8}$  cm.

But not only has direct measurement shown the radius of action to be one or two molecular diameters, but computations of it by Kleeman make it very close to this. For example, in ether Kleeman<sup>4</sup> computed the radius to be about  $3.4 \times 10^{-8}$  cm which is about the distance when the molecules are in contact. Van der Waals supposed it, indeed, to be only as long as this. Recently Einstein<sup>5</sup> has made a very interesting computation starting from the law of Eötvös, by which he shows, from thermodynamic reasoning, that the

<sup>1</sup> Johannot: *Phil. Mag.*, [5] 47, 501 (1899).

<sup>2</sup> Chamberlain: *Phys. Rev.*, 31, 170 (1910).

<sup>3</sup> Devaux: *Journal de Physique*, [5] 2, 699 (1912).

<sup>4</sup> Kleeman: "On the Radius of the Sphere of Action," *Phil. Mag.*, [6] 19, 840 (1910).

<sup>5</sup> Einstein: "Bemerkung zu dem Gesetz von Eötvös," *Annalen der Physik.*, [4] 34, 165 (1911).



range of cohesive action must be of the general value of, and proportional to, the distance between the molecular centers. This distance is of the order of magnitude in most liquids of  $10^{-8}$  cm. This result is so surprising that Einstein says of it: "This result appears at first very unlikely, for what should the radius of action of a molecule have to do with the distance between neighboring molecules? The supposition is only reasonable in case the neighboring molecules alone attract each other, but not those farther removed."

Sutherland<sup>1</sup> also came to the conclusion that the radius of action was about equal to  $v^{1/3}$ . We see then, that the evidence points to the conclusion that the radius of action of the molecular forces agrees very closely with  $v^{1/3}$ , the distance between the molecular centers, and varies directly with  $v^{1/3}$ . The only explanation of this fact appears to me to be that the attraction does not extend beyond neighboring molecules and, hence, must, in some way, be stopped by them.

Mills<sup>2</sup> alone, so far as I can find, has reopened the question whether the field is delimited by the surrounding molecules. Concluding, I believe erroneously, from an empirical law, that the attraction between molecules must vary inversely as the square of the distance, he was driven to the second conclusion that if this were the case the cohesion could not penetrate matter. He assumed, hence, that the surrounding molecules absorbed, or neutralized, the lines of cohesive force. The direct evidence and Einstein's reasoning leaves little doubt that the radius varies with the distance apart of the molecules and the only possible conclusion from this is that the surrounding molecules delimit the field as Mills supposes.

If it is a fact that the surrounding molecules delimit the field as the evidence indicates, cohesion is allied at once with magnetism, for this is the very supposition which Ewing made to explain some of the phenomena of magnetism. Each molecule of a ferromagnetic substance is supposed to be a

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<sup>1</sup> Sutherland: *Phil. Mag.*, [6] 4, 632, 636 (1902).

<sup>2</sup> Mills: *Jour. Phys. Chem.*, 15, 417 (1911).

magnet. In the non-magnetic state the magnetism of each molecule is supposed to be neutralized by the surrounding molecules, which have their magnetic axes variously directed. The magnetic field is thus limited to a single molecular diameter and will vary with the distance apart of the molecules. The magnetic field of each molecule is delimited by the surrounding molecules. If, however, these molecules are oriented, either by acting on each other, or by external forces, then the magnetic fields coincide and the magnetism may be perceived extending outward from the mass. In other words, to explain why magnetism does not persist in soft iron and to account for magnetic hysteresis, Ewing has made exactly the same assumption which has been made to explain why cohesion does not extend beyond molecular distances.

It seems to me not impossible that magnetism is the cohesive attraction of a molecule. If the molecule is of such a nature, or shape, that the total effect of the little magnets, its electron couples, coincide more or less completely so that the molecule has a polarity, and the molecules can be oriented in any way and held in position, we have the ferro and paramagnetic substances. If the molecules have many poles, so that there is no polarity of the molecules as a whole, there is a diamagnetic substance. The magnetic field of each molecule would be its cohesive field.

The recent work of Cotton and Mouton<sup>1</sup> on the orientation of molecules in the magnetic field seems to me to bear out such an interpretation. The work of Weiss<sup>2</sup> and Langevin<sup>3</sup> appears to point in the same direction, but Weiss, who has considered the possibility of the identity of cohesion and magnetism, states that he will shortly show that they can not be identical. Nevertheless it appears to me not impossible that magnetism is simply a special case of cohesion, and if this is true the rotation of the plane of polarized light by optically active substances would be easily understood.

<sup>1</sup> Cotton and Mouton: *Journal de Physique*, [5] 1, 40 (1911).

<sup>2</sup> Weiss: *Journal de Physique*, [5] 1, 900 (1911); [4] 6, 661 (1907).

<sup>3</sup> Langevin: *Ann. Chim. Phys.*, 5, 70 (1905).



Finally, if it is true that the surrounding molecules delimit the field of cohesion in any way whatever, and that only six molecules really take part in this delimitation, we can derive the value  $a/V^2$  of van der Waals' at once and very simply.

Suppose that each molecule has a certain mass of cohesion,  $M$ , and that two molecules attract each other directly as the product of their cohesive masses and inversely as the fourth power of the distance between their centers, then the attraction between two molecules would be  $M^2K/v^{4/3}$ , where  $v$  is the space at the disposal of a single molecule. Since each molecule attracts only the one above, below and to each side, the pressure per square centimeter of a double layer of molecules will be  $M^2K/v^{4/3}$  multiplied by the number of molecules in 1 sq. cm or  $1/v^{2/3}$ , making  $M^2K/v^2$ . Since the attraction extends only a single molecular diameter, we may multiply both numerator and denominator by  $N^2$ , where  $N$  is the number of molecules in the volume,  $V$ , and we obtain,  $N^2M^2K/N^2v^2 = M^2N^2K/V^2 = a/V^2$ .  $M^2K$  has already been shown to be  $2.98 \times 10^{-37}$  (Mol. Wt.  $\times$  Valences) $^{2/3}$ .

We have, as yet, no proof that only the six surrounding molecules are attracted, although Sutherland<sup>1</sup> has made a similar supposition. But such may be the case nevertheless. Einstein, in his calculation, computed that each molecule could be considered as lying at the center of a cube and that it attracted the 26 other molecules of the cube. Of course the fact that the value  $a/V^2$  may be so easily derived in this way does not furnish any proof that the attraction is inversely as the fourth power of the distance. But I know of no other derivation of  $a/V^2$  which involves so few, or less radical, assumptions.

The general conclusion of the paper is then, that cohesion,

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<sup>1</sup> Sutherland: Phil. Mag., [6] 17, 667 (1909). "The total potential energy of a number of like molecules is the same as if each caused its own domain to be uniformly electrized with an electric moment proportional to the linear dimensions of the domain, the direction of electrization being such that in general any molecule attracts its six immediate neighbors."

being a function of molecular weight and valence, is a function of the number of electron couples of the valences and atoms, and is, hence, probably of a magnetic nature. Magnetic substances may be supposed to be substances in which, owing to the orientation of the molecules, or to their polarity, or both these causes, the cohesive fields of the molecules are not delimited or neutralized by the surrounding molecules so that the cohesion attraction becomes apparent at more than molecular distances, and under such circumstances the substance is said to be magnetic.

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## THE INTERNAL PRESSURES OF LIQUIDS

BY ALBERT P. MATHEWS

The fundamental significance of the constant " $a$ " of van der Waals' equation makes its exact determination important.

Various methods have been proposed for the determination of this constant, but none of them are entirely satisfactory. In a recent paper<sup>1</sup> attention was drawn to a method by which it could be determined from the surface tension by the use of Thomas Young's formula,  $T = rK/3$ , combined with the law of Ramsay and Shields, and the values thus computed were shown to be closely similar for most substances to the values computed by van der Waals' method from the critical temperature and pressure, but in some cases they deviated considerably from his values. I found, also, that the values of the constant " $a$ " obtained by this method were simple functions of the products of the molecular weight and the number of valences in the molecule and that they could be computed from these values. Inasmuch as the method used in computing " $a$ " from the surface tension involved the value of the density at absolute zero, which was computed from Cailletet and Mathias' law of the rectilinear diameter, and involved, therefore, some uncertainty and was certainly too high, it was desirable to find a method of computing " $a$ " directly from the surface-tension measurements.

The desire of finding such a method was stimulated by the present great uncertainty of the value of the internal pressures of liquids. Traube<sup>2</sup> has within the past few years computed the internal pressure for many liquids, but the results he has obtained are, in my opinion, unreliable because his method of computation involves the use of the value " $b$ " the real molecular volume or co-volume, a very doubtful value. The values he finds for the internal pressure at zero degrees are, also, widely different from those computed by the use of " $a$ " found

<sup>1</sup> Mathews: Jour. Phys. Chem., 17, 154 (1913).

<sup>2</sup> Traube: Zeit. phys. Chem., 68, 291 (1909).

at the critical temperature; and in some cases they are not more than half those calculated recently by Lewis<sup>1</sup> from the latent heats of expansion of liquids.

Walden,<sup>2</sup> also, has recently calculated the value of "*a*" and the internal pressure from the surface tension. His calculation is, however, almost wholly empirical.

It is based, first, on Stefan's conclusion<sup>3</sup> that it takes one-half the work to move a particle into the surface which is required to carry it all the way to the vapor; and, second, upon an empirical relationship found by Walden between the surface tension and the molecular latent heat at the boiling point. It is, however, by no means certain that Stefan's conclusions are correct and his reasoning does not carry conviction. There is, also, probably an error in the assumption that the molecules do not change in size on passing from the liquid to the vapor and that the latent heat of vaporization represents only the work done in overcoming molecular cohesion. Finally Walden's values for "*a*" resemble Traube's. "*a*" is always much less than when computed by van der Waals' method from the critical data and much less than the values of Lewis. The values which Walden has obtained are about two-thirds the values given in this paper.

Values still smaller have been computed by Davies<sup>4</sup> from the latent heat of vaporization. The values he obtains are only about one-third those of Lewis. Winther<sup>5</sup> has still other results.

Many modifications of van der Waals' equation have been proposed in which "*a*" was considered variable with the temperature and "*b*" more, or less, constant. These attempts have not been fruitful. It is far more probable that "*b*," the volume correction, varies with temperature and volume and that "*a*," which is the "mass" factor of the cohesion, is

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<sup>1</sup> Lewis: *Phil. Mag.*, [6] 25, 61 (1912).

<sup>2</sup> Walden: *Zeit. phys. Chem.*, 66, 385 (1909).

<sup>3</sup> Stefan: *Wied. Ann.*, 29, 655 (1886).

<sup>4</sup> Davies: *Phil. Mag.*, [6] 24, 422 (1912).

<sup>5</sup> Winther: *Zeit. phys. Chem.*, 60, 603 (1907).



constant. "*a*," indeed, as van der Waal's has repeatedly shown, should be considered constant unless association, or quasi-association, occurs. "*a*" contains the factor  $N^2$ ,  $N$  being the number of molecules in the volume,  $V$ , hence any association will lower "*a*" by this factor.

The wide divergence of these various values proposed for the internal pressure is shown in Table I expressing the internal pressure in atmospheres at zero degrees, except in the case of Walden where the values are for the boiling points and Davies for 15° C.

TABLE I

Substance	Davies 15°	Traube 0°	Walden b. p.	v. d. Waals 0°	Lewis 0°	Winther
Benzene	1102	1380	1570	2494	2639	1792
Toluene	1188	1180	1340	2228	2847	—
Cymene	661	—	—	—	2718	—
Ether	778.9	990	{ 1150 } { 1210 }	1723	1932	1220
CCl <sub>4</sub>	1076	1305	1490	2205	2518	1680
CS <sub>2</sub>	1683	1980	2170	3363	2917	—
Et acetate	—	2210	{ 1280 } { 1340 }	—	2466	1486

In this paper are given the values of "*a*" obtained in several quite different ways, all of which yield closely agreeing results.

1. The first method is a computation from the surface tension. The assumption involved in this method is the depth of the surface film expressed in the number of molecular layers. That the assumption is correct is proved by the outcome.

2. The second method is a modification of Thomas Young's method combined with the law of Eötvös as developed in my former paper, but with certain corrections.

3. In the third method "*a*" is computed from van der Waals' equation at the critical temperature, the assumption being made that in all normal substances  $b_c = 2V_o = 2V_c/S$ ,  $S$  being the critical coefficient and equal to  $RT_c/V_cP_c$ .

4. In the fourth method "*a*" is computed from the internal

latent heat of vaporization close to the critical temperature. The only assumption made here is that Mills' or Dieterici's formula for the internal latent heat is more correct close to the critical temperature than the internal latent heat computed by Biot's formula.

5. Finally "*a*" is computed from the number of valences and the molecular weight by the formula:  $a = C(M \times \text{Val})^{2/3}$ .

### 1. Computation of "*a*" from the Surface Tension

The surface film is determined by the difference of cohesive attraction in the vapor and liquid. The surface energy must, hence, be a function of the difference in cohesive energy in the liquid and vapor, or of the expression  $a(1/V_l - 1/V_v)$ . This cohesive energy has been lost in passing the liquid through the surface layer which separates the two states and should be calculable from the surface tension.

Suppose we have a sphere of a gram mol of a liquid in contact at all points with its saturated vapor. Its density is uniform except in the surface film where it decreases by a series of steps from liquid to vapor density. The surface film may be conceived as a series of concentric shells, each a molecular diameter thick, and each outer one less dense than the inner until the state of uniform vapor density is reached. Furthermore, on passing from one of these molecular shells lying within to the one next beyond it, always the same amount of cohesive energy will be lost, if the density diminishes uniformly from shell to shell.

The surface tension *T*, is the tension along a line 1 cm. in length in the surface film and one molecular layer deep. It represents the force necessary to stretch the surface, that is to rupture one of these shells, and thus drag one, or more, molecules from the inner core of uniform density into the first concentric shell; and of course the force necessary to drag molecules from the first shell to the second, and from the second to the third and so on, since the same force is necessary to drag molecules from each inner shell to the next outer. This tension, *T*, is numerically equal, also, to the surface tension



energy per square cm. of the surface film. For each concentric shell of the surface one molecular diameter deep, the surface energy is, then,  $T$  times the surface, or  $TV_1^{2/3}$ ; and the total energy in the surface or  $\sigma$ , will be equal to this amount multiplied by the number of shells, which we shall represent by the letter  $n$ , thus we have the equation:

$$(1) \quad \sigma = TV_1^{2/3}n$$

If now a gram mol of liquid passes from liquid to vapor it must pass through this surface in which surface energy is gained at the expense of the cohesive energy which is lost. To determine the total amount of energy which is thus changed in passing the whole gram mol through the surface, it is only necessary to determine how many times we shall have to make a new surface shell until the whole of the gram mol has passed through. Since the total number of molecules in the gram mol is  $N$ , and there are in a surface shell  $N^{2/3}$  molecules, we shall have to make a new surface  $N/N^{2/3}$  times, or  $N^{1/3}$  times. The total energy then which will be gained as surface tension energy will be  $\Sigma$ , or

$$(2) \quad \Sigma = TV_1^{2/3}N^{1/3}n.$$

This same value may be obtained, also, in the following way: The surface tension measures the force necessary to overcome the surface tension pressure along a line 1 cm long and a molecular diameter deep. The surface-tension pressure per sq. cm acting in the plane of the surface is hence  $T/v^{1/3}$ ,  $v$  being the volume of a single molecule. Multiplying numerator and denominator by  $N^{1/3}$  we have the pressure per square cm  $N^{1/3}T^{1/3}/V_1^{1/3}$ . If this pressure work through the volume  $V_1$ , the volume of one gram mol, we have the surface tension energy if a whole gram mol were present as a surface shell, or  $N^{1/3}TV_1^{2/3}$ . Multiplying this by the number of shells, or  $n$ , we have our former expression.

Equation (2) contains the unknown factor " $n$ ," that is the number of layers one molecular diameter thick constituting the surface film. Since I knew of no way of measuring this,

the following assumption was made based on van der Waals' conclusion that the surface film is infinitely thick at the critical temperature. At absolute zero, where the molecules are presumably in contact, it may be assumed that the surface film is only a single molecular diameter deep. At the critical temperature, on the other hand, it must be infinitely deep. That is, no matter how many layers of molecules one passes over, one can never, at that temperature, get to a region of differing density. Between absolute zero and the critical temperature the depth of the surface film must lie between these two values, increasing with the temperature, and presumably in all normal substances at corresponding temperatures it will be the same number of molecular layers thick. I accordingly made the guess that it would be equal to  $(T_c/(T_c - T))^{2/3}$  molecular diameters since this fraction is equal to  $(d_o/(d_1 - d_v))^2$  (see page 617). This guess turned out to be correct if Eötvös surface-tension figures are used, but if Ramsay and Shields' are taken the fraction must be raised to the 0.76 power and even then the value of " $a$ " computed by this assumption runs down near the critical temperature. Since Eötvös measured the surface tension by a method which entirely avoided any assumption as to the angle of contact and Ramsay and Shields used the capillary method, which involves such an assumption, I believe Eötvös figures and his statement of the law is to be preferred. That his formula of  $TV_1^{2/3} = 2.27 (T_c - T)$  is to be preferred on other accounts is shown by the calculations which follow:

The total surface energy gained by passing a gram mol through the surface is hence:

$$(3) \quad \Sigma = TV_1^{2/3}N^{1/3}(T_c/(T_c - T))^{0.76} \text{ ergs; or}$$

$$(4) \quad \Sigma = TV_1^{2/3}N^{1/3}(T_c/(T_c - T))^{2/3} \text{ ergs.}$$

Formula (4) is to be preferred, when the surface tension is measured by methods which do not involve the angle of contact.

We only have left to find the relation between the amount of energy thus lost and the difference in the cohesive energy



in a gram mol of liquid and vapor, respectively. I think that the total surface-tension energy must be one-third of the total difference in cohesive energies in equal weights of the two phases separated by the surface. The energy in the surface is an expression of the difference in cohesive pressure in one direction only, whereas the two phases differ in their cohesion in three dimensions. That the value one-third is correct is shown by the computations which follow. The value one-third was that adopted also by Young more than a century ago, but his reasoning is so condensed that it is hard to follow. His statement is as follows:<sup>1</sup>

"Upon these grounds we may proceed to determine the actual magnitude of the contractile force derived from a given cohesion extending to a given distance. Supposing the corpuscular attraction equable throughout the whole sphere of its action, the aggregate cohesion of the successive parts of the stratum will be represented by the ordinates of a parabolic curve; for at any distance  $x$  from the surface, the whole interval being  $a$ , the fluxion of the force will be as  $dx(a - x)$ , since a number of particles proportional to  $dx$  will be drawn downwards by a number proportional to  $a$ , and upwards by a number proportional to  $x$ , and the whole cohesion at the given point will be expressed by  $ax - x^2/2$ ; and this at last becomes  $a^2/2$ , which must be equal to the undiminished cohesion in the direction of the surface. Consequently the difference of the forces acting on the sides of the elementary cube will everywhere be as  $a^2/2 - ax + x^2/2$  and the fluxion of the whole contractile force will be  $dx(a^2/2 - ax + x^2/2)$ , the fluent of which when  $x = a$  becomes  $a^3/6$ , which is  $1/3$  of  $a \times a^2/2$ , the whole undiminished cohesion of the stratum." "We may, therefore, conclude, in general, that the contractile force is one-third of the whole cohesive force of a stratum of particles equal in thickness to the interval to which the primitive equable cohesion extends," or  $T = aK/3$ .

Accepting this coefficient of  $1/3$  of Young in place of that of

<sup>1</sup> Young, T: "Article on Cohesion," collected works, p. 460.

$1/2$  of Stefan, or  $3/20$  as computed by Lord Rayleigh, we have the complete formula

$$(5) \quad a(1/V_1 - 1/V_v)/3 = TV_1^{2/3}N^{1/3}(T_c/(T_c - T))^{2/3}.$$

Changing to density in place of volume on the left hand side we have

$$(6) \quad a = 3TV_1^{2/3}N^{1/3}M(T_c/(T_c - T))^{2/3}/(d_1 - d_v);$$

or if Ramsay and Shields' surface-tension figures are used

$$(7) \quad a = 3TV_1^{2/3}N^{1/3}M(T_c/(T_c - T))^{0.76}/(d_1 - d_v).$$

The result is given in dynes for gram mol quantities.  $M$  is the molecular weight;  $d_1$  and  $d_v$ , liquid and vapor density, respectively;  $N$ , the number of molecules in a gram mol, is  $6.21 \times 10^{23}$ ;  $T$  is the surface tension in dynes;  $V_1$ , the volume of a gram mol at temperature,  $T$ .

From the foregoing it appears that  $1/3$  of the internal latent heat of vaporization is due to the cooling caused by the increase of the surface brought about by the transfer of molecules from the region of uniform density into the surface, and their passage through the surface.

In Table 2, I have given the calculation of " $a$ " by formula (7) for a number of substances using Ramsay and Shields', or Ramsay and Acton's or Renard and Guye's surface-tension determinations and the densities from S. Young's<sup>1</sup> recent work.

In Table 2 the constancy of " $a$ " is shown to be good for normal substances, except near the critical temperature where it generally falls off somewhat. This may be due to the inaccuracy of the surface tension close to the critical temperature, but it is more probably due to the fact that the thickness of the surface layer in molecular diameters is not properly represented by the expression  $(T_c/(T_c - T))^{0.76}$  and possibly to the influence of the angle of contact.

There is in general a tendency of the value of " $a$ " to rise except near the critical point, and this tendency is most pronounced in an associating substance such as ethyl alcohol.

The agreement is admirably clear to the critical temperature

<sup>1</sup> Young, S: Proc. Roy. Dublin Soc., 12, 374 (1910).



if the computation is made by Eötvös formula, for  $TV_e^{2/3}$  in the manner shown farther on (Table 6).

I may repeat, to make the point quite clear, that I think by taking the value  $(T_c/(T_c-T))^{0.76}$  in place of the theoretical value  $(T_c/(T_c-T))^{2/3}$  for these surface-tension measurements we offset, at least approximately, some constant source of error, possibly the angle of contact, involved in the determination of the surface tension by the capillary method. The opinion has been expressed by others that the error due to the neglect of the angle of contact, or the assumption that it is zero, will increase with the temperature.

TABLE 2

"a" in dynes for a gram mol computed by formula (7)

Methyl formate		Ethyl acetate		Benzene	
$t^\circ$	$a \times 10^{-13}$	$t^\circ$	$a \times 10^{-13}$	$t^\circ$	$a \times 10^{-13}$
20	1.217	+20	2.338	11.2	2.214
40	1.224	80	2.366	46	2.220
60	1.228	100	2.366	80	2.220
80	1.233	120	2.370	120	2.235
100	1.237	140	2.367	160	2.242
120	1.237	160	2.368	200	2.242
140	1.231	180	2.368	240	2.225
160	1.218	200	2.353	260	2.179
180	1.193	220	2.355	270	2.081
200	1.058	240	2.193	280	1.521
210	0.885	245	2.107	288.5	$T_c$
214	$T_c$	250.1	$T_c$	—	—
Chlorbenzene		Carbon tetrachloride		Ether	
9.5	2.944	11.8	2.393	—89.2	2.038
45.6	2.947	46	2.404	+20	2.044
77.1	2.962	80	2.387	40	2.039
150	2.958	120	2.405	60	2.072
180	2.965	140	2.411	70	2.023
200	2.975	160	2.422	80	2.021
220	2.977	200	2.431	100	2.025
260	2.984	220	2.401	110	2.023
310	2.977	240	2.344	120	2.020
359.2	$T_c$	260	2.187	140	1.990
—	—	270	2.483	160	1.918
—	—	283	$T_c$	193	2.745
—	—	—	—	193.8	$T_c$

TABLE 2—(Continued)

Methyl butyrate		Cymene		Anisol		Toluene	
<i>t</i>	$a \times 10^{-13}$	<i>t</i>	$a \times 10^{-13}$	<i>t</i>	$a \times 10^{-13}$	<i>t</i>	$a \times 10^{-13}$
10	2.706	11.9	4.982	11.1	3.424	13.1	2.744
46.2	2.964	31.7	5.013	33.5	3.452	29.1	2.788
78.2	3.005	74.5	5.026	88	3.471	78.2	2.827
100	3.040	108.9	5.055	119	3.486	132.5	2.860
132.5	3.110	134.9	5.075	147.9	3.498	—	—
185	3.073	163.4	5.143	—	—	—	—
210	3.049	—	—	—	—	—	—
238	3.093	—	—	—	—	—	—
281.3	$T_c$	—	—	—	—	—	—
Piperidine		$CS_2$		$CHCl_3$		Methyl acetate	
15.2	2.638	9.7	1.277	10.2	1.837	10	1.719
46.6	2.664	46	1.222	45.5	1.860	46.2	1.727
78.4	2.672	61	1.434	77.6	1.874	78.3	1.746
132.5	2.791	—	—	—	—	132.4	1.777
—	—	—	—	—	—	185	1.770
—	—	—	—	—	—	200	1.740
—	—	—	—	—	—	215	1.720
—	—	—	—	—	—	232.7	$T_c$
Ethylene dibromide		Methyl isobutyrate		Ethyl alcohol		Ethyl propionate	
12.2	2.776	10	2.871	20	0.951	10	2.903
44.9	2.842	46.2	2.894	40	0.966	46.2	2.932
77.2	2.912	78.2	2.920	100	1.055	78.2	2.969
131.3	3.074	100	2.932	150	1.157	100	3.004
—	—	132.2	2.937	180	1.235	132.2	3.061
—	—	185	2.941	220	1.485	185	3.047
—	—	210	2.947	—	—	210	3.060
—	—	237.6	2.913	—	—	237.6	3.010
—	—	267.7	$T_c$	—	—	272.9	$T_c$
Propyl acetate		Methyl propionate		Propyl formate		Ethyl iodide	
10	2.917	10	2.287	10	2.270	19.1	2.034
46.2	2.954	46.2	2.302	46.2	2.278	78.2	2.044
78.2	2.966	78.2	2.329	78.2	2.331	281	$T_c$
100	2.981	132.6	2.350	85	2.334	Metaxylene	
132.6	3.010	184.9	2.365	131.7	2.356		
185	3.011	237.7	2.278	185	2.348	10	3.408
210	2.999	250	1.962	210	2.339	—	—
238.2	2.980	257.4	$T_c$	237	2.312	—	—
276.2	$T_c$	—	—	264.85	$T_c$	—	—



## 2. Derivation of "a" from the Surface Tension by Eötvös Law

By the law of Eötvös the surface-tension energy  $TV_1^{2/3}$  is equal to  $C(T_c - T)$ . The surface-tension energy is a linear function of the temperature counting downward from the critical temperature. The derivation of  $TV_1^{2/3}$  and  $N^{1/3}TV_1^{2/3}$  has already been given on page 607.

a. What is C of Eötvös?

Eötvös found that C varied between 2.27 and 2.34 as is shown in Table 4, but he believed the variation to be accidental and that C should be constant for all substances. It has since been shown that C is not constant. What C is may be shown as follows:

Since the surface energy decreases uniformly with an increase of the kinetic energy of the molecules, and is accordingly a linear function of the temperature, one of the constituents of C must be the gas constant R; and since there are only  $N^{2/3}$  molecules in the surface, where N is the number in a gram mol, R must be R for a gram mol divided by  $N^{1/3}$ . The remainder of C should be  $1/3$  the ratio of the internal to the external pressure at the critical temperature as that is the point of departure. Young has shown that the surface-tension pressure is  $1/3$  the cohesive pressure; and the greater the external pressure the less important the internal pressure will be. Hence C, I thought, must equal  $K_c R / 3P_c N^{1/3}$ . While the foregoing reasoning was not entirely convincing the result turned out to be correct, I believe, as will presently be shown. I have uniformly taken N as  $6.21 \times 10^{23}$  and R as  $8.321 \times 10^7$ .

$$(8) \quad TV_1^{2/3} = K_c R (T_c - T) / 3P_c N^{1/3}.$$

Since  $K_c = a/V_c^2$  we have

$$(9) \quad TV_1^{2/3} = aR(T_c - T) / 3V_c^2 P_c N^{1/3}$$

$$(10) \quad a = 3T_c V_c N^{1/3} TV_1^{2/3} / (T_c - T) S,$$

since  $RT_c/V_c P_c = S$ , and  $R = SV_c P_c / T_c$ .

Formula (10) gives the second method of computing "a."

The following values in Table 3 were computed by formula (10) from Schiff's surface-tension figures at the boiling point.  $a$  is in dynes per gram mol.

TABLE 3

Substance	S	$a \times 10^{-12}$
Methyl acetate	3.940	15.90
Propyl acetate	3.933	28.14
Diisobutyl	3.811	40.01
Benzene	3.754	20.52
Ether	3.810	19.12
Hexane	3.831	27.22
CCl <sub>4</sub>	3.676	22.60

These results are uniformly somewhat lower than those computed from Ramsay and Shields' figures by formula (7).

Returning to the value  $C$  of Eötvös formula, the following computation shows that it has in it the components ascribed to it.

It was believed, for the reasons given, that  $C$  of Eötvös must be equal to  $K_c R / 3 P_c N^{1/3}$ . The ratio of the internal to the external pressure is supposed to be, at the critical temperature, very approximately equal to 7. Its real value is as follows: If we assume that  $b_c$  of van der Waals' equation is always twice the volume at absolute zero, then  $b_c = 2V_o$ . Since  $V_o = V_c/S$ ,  $b_c = 2V_c/S$ . If this is so then the ratio between  $K_c$  and  $P_c$  is equal to  $(S^2 - S + 2)/(S - 2)$ .  $S = RT_c/V_c P_c$ . Hence  $C$  of Eötvös must be as follows:

$$(11) \quad C = (S^2 - S + 2)R/(S - 2)3N^{1/3}.$$

Formula (11) can now be tested since  $C$  is known to lie between 2.27 and 2.34 for several non-associating substances. The results of a computation of  $C$  by this formula and the values given by Eötvös are compared in Table 4.

The results are evidently closely similar, but unfortunately Eötvös did not give the value of  $C$  for many substances of which  $S$  is known.



TABLE 4

Substance	S	C computed	C found by Eötvös
Methyl acetate	3.943	2.273	—
Methyl butyrate	3.907	2.275	—
Ethyl alcohol	4.025	2.270	—
Pentane ( <i>n</i> )	3.761	2.285	—
Ether	3.806	2.280	2.280 (6–62°); 2.26 (62–120°)
Benzene	3.794	2.279	—
Oxygen	3.40	2.355	—
Carbon dioxide	3.44	2.344	2.280
Propyl acetate	3.933	2.280	<del>2.287</del>
Carbon bisulfide	—	—	2.37
Chloroform	—	—	2.30
Ethylene bromide	—	—	2.27

## 2. The Computation of “*a*” from Thomas Young’s Formula

As pointed out in an earlier paper, the first method proposed for computing the internal pressure was Young’s, namely:

$$(12) \quad T = rK/3 = ra/V^{2/3}$$

*r* being the radius of action. If *r* is taken at absolute zero as equal to  $v^{1/3}$  we have finally

$$(13) \quad T v_o^{2/3} = M^2 K / 3 v_o$$

$M^2 K$  is the factor “*a*” for a single molecule and  $v_o$  the volume of a single molecule at absolute zero. In my former paper  $v_o$  was assumed for all except the simple gases to be  $v_c/4$ , and for those gases it was taken as  $v_c/3.6$ . Timmermans<sup>1</sup> has recently confirmed S. Young’s finding that the rectilinear diameter law gives too high values for the density at low temperatures and that van der Waals is correct in taking the density at absolute zero as *S* times the critical density, where *S* is the critical coefficient, or  $RT_c/V_c P_c$ , which varies with different substances between 3.4 and 4.  $T v_o^{2/3}$  I formerly computed by Ramsay and Shields’ equation assuming that it

<sup>1</sup> Timmermans: Proc. Roy. Dublin Soc., [N S] 13, 310 (1912).

held at low temperatures and that  $Tv_o^{2/3}$  was equal to  $2.19-(T_c-6)/N^{2/3}$  dynes. This, however, gives values uniformly lower than method 1 and I have accordingly reverted to Eötvös original formula as already stated according to which

$$(14) \quad TV_1^{2/3} = C(T_c - T).$$

The theoretical surface-tension energy at absolute zero should be, then,  $CT_c$  ergs for a gram mol, or  $CT_c/N^{2/3}$  ergs for a single molecule. Substituting in (13) we have

$$(15) \quad M^2KS/3v_c = CT_c/N^{2/3}.$$

Changing to the total volume  $V$ , in place of the volume of a single molecule, we have:

$$(16) \quad a = N^2M^2K = 3CN^{1/3}V_cT_c/S.$$

Since  $S = RT_c/P_cV_c$  by substitution in the foregoing we have:

$$(17) \quad a = 3CN^{1/3}P_cV_c^2/R \text{ ergs.}$$

The value of  $C$  has been given in (11). It can be found also by comparing (17) with (29) which follows. Substituting its value we have:

$$(18) \quad a = (S^2 - S + 2)P_cV_c^2/(S - 2)$$

which is identical with the formula derived from van der Waals' equation on the basis that  $b_c = 2V_c/S$ . Young's formula, then, at absolute zero yields the same result as the others when combined with Eötvös, if, however, we make no assumption as to  $C$ , but take it, as Eötvös thought it should be as constant for all substances, we obtain the approximately correct value of " $a$ " for all except very simple substances:

$$(19) \quad a = 3 \times 2.27N^{1/3}V_cT_c/S.$$

Computations of " $a$ " by formula 18 and 19 are given in Table 9.

We may check the foregoing reasoning in the following way avoiding any assumption of what fraction of  $V_c b_c$  is. Taking Young's formula:  $T = rK/3$ ,  $r$  being equal to  $v^{1/3}$  at absolute zero, multiplying both sides by  $v_o^{2/3}$  and then by  $N$  we have:



$$(20) \quad N^{1/3}TV_o^{2/3} = V_oK_o/3.$$

Dividing by  $T_c$  and remembering that by (8)

$$N^{1/3}TV_o^{2/3}/T_c = K_cR/\bar{P}_c$$

$$(21) \quad K_oV_o/3T_c = K_cR/3P_c.$$

As  $K_o = a/V_o^2$ , and  $K_c = a/V_c^2$  and  $V_o = V_c/S$

$$(22) \quad 1/V_oT_c = R/V_c^2P_c.$$

Therefore

$$(23) \quad S = RT_c/P_cV_c.$$

Formula 23 is true.

We may also check our reasoning as follows: From formula (6)  $a = 3MN^{1/3}TV_o^{2/3}(T_c/(T_c - T))^{2/3}/(d_1 - d_v)$  and from (8)  $3N^{1/3}TV_o^{2/3} = K_cR(T_c - T)/P_c$  we have

$$(24) \quad a = K_cRM(T_c - T)^{1/3}T_c^{2/3}/P_c(d_1 - d_v),$$

hence

$$(25) \quad d_1 - d_v = RM(T_c - T)^{1/3}T_c^{2/3}/V_c^2P_c.$$

The values of  $d_1 - d_v$  computed by (25) for ethyl acetate compare as shown in Table 5 with those found by S. Young.

TABLE 5—ETHYL ACETATE,  $d_1 - d_v$

$t$	Computed	Found by S. Young
$-83.4^\circ$	1.078	1.022 (Timmermans)
0	0.9490	0.9244
100	0.8007	0.7910
200	0.5555	0.5630
240	0.3356	0.3279
249	0.2071	0.1551
249.1 ( $T_c$ )	0.0000	0.0000

At absolute zero (25) becomes  $d_o = RMT_c/V_c^2P_c = Sd_c$  which is correct.

Substituting this value in (25) we have

$$(26) \quad d_1 - d_v = d_o((T_c - T)/T_c)^{1/3}.$$

$d_o$  computed by (26) for normal pentane using Timmerman's data for densities below zero and S. Young's above is as follows:

$t$	$d_o$	$t$	$d_o$
—136.5	0.8611	—35.3	0.8580
—123.3	0.8581	—13.1	0.8641
—116.2	0.8578	— 6.2	0.8603
—111.6	0.8576	0	0.8603
—104.85	0.8570	40	0.8685
— 74.25	0.8556	80	0.8776
— 73.95	0.8570	100	0.8818
— 63.3	0.8571	140	0.8882
— 53.6	0.8566	180	0.8832
— 45.0	0.8571	190	0.8762
		197	0.8443
		197.2	$T_c$

$d_o$  required by the formula:  $d_o = Sd_c$ , with Young's value of  $d_c$ , is 0.8736.

The formula appears then to be correct.

By substituting in (6) the value of  $TV_1^{2/3}$  found by combining (11) and (14) there is obtained

$$(27) \ a = M((S^2 - S + 2)/(S - 2))R(T_c - T)^{1/3}T_c^{2/3}/(d_1 - d_v).$$

Table 6 contains the results of calculating "a" by this formula.

TABLE 6—"a" BY FORMULA (27) IN DYNES FOR A GRAM MOL

Isopentane		Pentane		Oxygen (Mathias and Onnes)	
$t$	$a \times 10^{-12}$	$t$	$a \times 10^{-12}$	$t$	$a \times 10^{-12}$
—136.5	22.52	—123	23.15	—210.4	1.966
— 30.6	22.63	— 35.3	23.30	—182	1.956
0	22.54	0	23.03	—154.51	1.966
50	22.30	50	22.84	—140.2	1.966
100	22.00	100	22.70	Ether	
150	21.83	150	22.38		
170	22.01	180	22.48	—89.2	20.53
180	22.25	190	22.66	+ 20	20.34
187	22.73	195	22.81	100	19.95
187.8	$T_c$	197	23.51	150	19.81
		197.2	$T_c$	180	19.90
				190	19.85
				193.8	$T_c$



This formula gives remarkably constant results for the calculation clear to the critical temperature from nearly the point of solidification. The results are in agreement with other methods of calculating "a" already given or to be described. The density values for all except oxygen are those of Timmermans or Young.

### 3. Computation of "a" from van der Waals' Equation at the Critical Temperature assuming that $b_c = 2V_o = 2\bar{V}_c/S'$

The values for "a" computed by the preceding methods from the surface tension agree with those computed from van der Waals' equation at the critical temperature assuming that  $b_c$ , the co-volume, or the real volume of the molecules, is always in all normal substances just twice the volume at absolute zero, or that  $b_c = 2V_o$ . As the volume at absolute zero is equal to the critical volume divided by S, where  $S = RT_c/V_cP_c$ ,  $b_c = 2V_c/S$ . Applying this to the equation, since  $V_cP_cS = RT$ , we have

$$(28) \quad a = (S^2 - S + 2)T_c^2R^2/(S^2(S - 2)P_c); \text{ or}$$

$$(29) \quad a = (S^2 - S + 2)P_cV_c^2/(S - 2).$$

Formula (28) which corresponds to van der Waals' formula:  $a = 27T_c^2/(64 \times 273^2 \times P_c)$ , has been used in computing "a" in Table 8. The values thus obtained are practically identical with those computed from the surface tension.

Formula (29) has already been obtained as (18).

### 4. The Computation of "a" from the Internal Latent Heat of Vaporization

That the foregoing values of "a" are correct is proved by their agreement with the values computed from the internal latent heat of vaporization close to the critical temperature.

If all the internal latent heat,  $\lambda$ , was used in overcoming molecular cohesion, then the equation should hold

$$(30) \quad \lambda = L - E = a(1/V_1 - 1/V_v),$$

where L is the total latent heat and E the external work. This equation does not hold except close to the critical tem-

perature, since as we go to lower temperatures " $a$ ," computed by this formula, becomes steadily larger. This is owing to the fact that some of the latent heat is consumed in doing other things than in overcoming molecular cohesion, a part probably being rendered latent by an actual expansion of the molecules.  $\mu$  is the latent heat used in increasing the intramolecular energy. It vanishes close to the critical temperatures. I have, therefore, computed " $a$ " by this formula within a degree or so of the critical temperature. At temperatures lower than this " $a$ " by this method will be found too large, but within a fraction of a degree of the critical temperature the change in the size of the molecules is probably negligible. To find  $\lambda$  at this temperature I have computed it from S. Young's recently published data of the liquid and vapor densities using Mills'<sup>1</sup> formula for the computation of  $\lambda$ , namely,

TABLE 7—COMPUTATION OF " $a$ " FOR ONE GRAM MOL FROM INTERNAL HEAT (NEGLECTING  $\mu$ )

Substance	Distance from the critical temperature	$a \times 10^{-13}$
Pentane ( $n$ )	—0.05°	2.108
Pentane (iso)	—0.4	2.065
Hexane	—0.8	2.814
Heptane	—3.5	3.616
Octane ( $n$ )	—6.2	4.575
Hexamethylene	—1.0	2.445
Ether	—0.8	1.945
Carbon tetrachloride	—3.15	2.202
Benzene	—0.15	2.112
Fluorbenzene	—6.55	2.266
Chlorbenzene	—89.2	3.260
Methyl formate	—0.5	1.145
Methyl acetate	—0.7	1.772
Methyl propionate	—1.4	2.349
Ethyl acetate	—1.0	2.359
Propyl formate	—4.85	2.395
Ethyl propionate	—2.9	3.058
Methyl butyrate	—1.3	2.989
Methyl isobutyrate	—1.05	2.892

<sup>1</sup> Mills: Phil. Mag., 21, 85 (1911).



$\lambda = C(d_1^{1/3} - D_v^{1/3})$ . The value of  $C$  was the mean  $C$  taken from Mills' recent paper. These values of the internal latent heat are very similar to those obtained by Dieterici's formula  $\lambda = CRT \ln d/D$ . If the internal latent heat is computed using the vapor pressures computed by Biot's formula close to the critical temperature too low values are obtained as Mills has pointed out.

The values for " $a$ " for one gram mol computed from near the critical temperature are given in Table 7. Column 2 of that table shows how many degrees below the critical temperature data were taken for the computation. The nearer the critical temperature the more reliable the data should be.

$$(31) \quad a = M(\lambda - \mu)/(d_1 - d_v).$$

It is interesting now to see how large  $\mu$  is relative to  $\lambda$  at different temperatures; that is, how much of the heat of vaporization is rendered latent by the expansion of the molecules, or by an increase in their rotatory energy. By formula (31)  $a = M(\lambda - \mu)/(d_1 - d_v)$ ; and by (29)  $a = (S^2 - S + 2)P_c V_c^2 / (S - 2)$ . Hence we have

$$(32) \quad \lambda - \mu = (S^2 - S + 2)P_c V_c^2 (d_1 - d_v) / M(S - 2).$$

But it was found by Mills that  $\lambda = C(d_1^{1/3} - d_v^{1/3})$  so that

$$(33) \quad \mu = C(d_1^{1/3} - d_v^{1/3}) - (S^2 - S + 2)P_c V_c^2 (d_1 - d_v) / M(S - 2).$$

The calculation of  $\mu$  for one gram mol of pentane by formula (33) resulted as follows:

$t$	$\mu \times 10^{-10}$ ergs
$-273^\circ$	4.25
+ 30	4.46
60	3.51
100	2.18
150	0.62
190	-0.0026
197.2	$T_c$

At  $30^\circ$ , therefore, the total latent heat for one gram is 85.76 cal; the total internal latent heat, or  $\lambda$ , is 78.80 cal.;

TABLE 8— $a \times 10^{-12}$  FOR ONE GRAM MOL IN DYNES

I Substance.	$2^1$	3	4	5	6	$7$ Valence <sub>q</sub>
Oxygen	> 1,700(?)	1.966	2.002	—	2.014	O = 1
Hydrogen (S = 3.4)	—	0.306	0.317	—	0.317	N = 1
Nitrogen	—	1.796	1.836	—	1.842	N = 1; O = 4
Nitrous oxide	—	5.070	5.249	—	5.180	N = 1; O = 4
Pentane ( <i>n</i> )	—	22.52	22.43	21.08	21.96	N = 1; O = 4
Pentane (iso)	—	22.07	22.17	20.65	21.96	N = 1; O = 4
Hexane	—	—	—	28.14	27.71	N = 1; O = 4



TABLE 8—(Continued)

Heptane	—	34.82	34.82	<36.16	33.80	
Octane	44.72	42.00	41.98	<45.75	40.17	
Ether	20.33	20.13	20.21	19.45	20.46	Cl = 3
Carbon tetrachloride	24.01	24.28	24.53	<22.02	22.94	
Benzene	22.33	22.32	22.41	21.12	22.19	Cl = 3
Chlorobenzene	29.73	30.00	30.09	<32.60	29.95	
Toluene	28.12(?)	—	—	—	27.97	
Metaxylene	34.08	—	—	—	34.06	
Anisol	34.66	—	—	—	32.26	
Cymene	49.82(?)	—	—	—	47.09	Cl = 3
Chloroform	18.57	—	—	—	18.00	
Methyl formate	12.24	12.45	12.44	11.45	12.25	
Methyl acetate	17.43	17.03	17.01	17.72	17.42	
Methyl propionate	22.83	22.15	22.16	<23.49	22.96	
Ethyl acetate	23.61	22.08	22.07	<23.59	22.96	
Propyl formate	23.21	23.05	23.05	<23.95	22.96	
Methyl butyrate	30.00	28.08	28.06	<29.89	28.84	
Methyl isobutyrate	28.94	27.55	27.60	<28.92	28.84	
Propyl acetate	29.78	28.05	28.03	—	28.84	
Ethyl propionate	29.98	27.89	27.87	<30.58	28.84	Br = 3
Ethylene dibromide	29.01(?)	—	—	—	28.36	
Piperidine	26.91(?)	—	—	—	25.54	S = 6
Carbon bisulfide	13.38	—	—	—	14.34	
Ethyl alcohol	12.53(?)	12.44	12.44	—	10.26	Cl = 3
Stannic chloride	—	32.43	32.58	—	32.58	

<sup>1</sup> The figures of column 2 are the average values of Table 2, omitting divergent values close to critical temperatures.

TABLE 9— $a \times 10^{-12}$  COMPUTED BY VARIOUS FORMULAS

$\frac{1}{n}$	2	3	4	5	6	7	8	9	10
Oxygen	> 1.700	—	—	2.002	1.966 (-210.4°)	2.002	1.800	—	2.014
Pentane (n)	—	—	—	22.43	22.70 (100°)	22.43	22.13	21.08	21.96
	$a = \frac{3}{2} T V_{\frac{2}{3}}^{\frac{1}{3}} M^{\frac{1}{3}} N^{\frac{1}{3}} / (T^{\frac{1}{3}} / (T^{\frac{1}{3}} - T^{\frac{1}{3}})) (d^{\frac{1}{3}} - d^{\frac{1}{3}})$	$a = \frac{3}{2} T V_{\frac{2}{3}}^{\frac{1}{3}} M^{\frac{1}{3}} N^{\frac{1}{3}} / (T^{\frac{1}{3}} / (T^{\frac{1}{3}} - T^{\frac{1}{3}})) (d^{\frac{1}{3}} - d^{\frac{1}{3}})$	$a = \frac{3}{2} T V_{\frac{2}{3}}^{\frac{1}{3}} M^{\frac{1}{3}} N^{\frac{1}{3}} / (T^{\frac{1}{3}} / (T^{\frac{1}{3}} - T^{\frac{1}{3}})) (d^{\frac{1}{3}} - d^{\frac{1}{3}})$	$a = ((S^2 - S + 2) / (S - 2)) P V^{\frac{2}{3}}$	$a = M((S^2 - S + 2) / (S - 2)) R(T^{\frac{2}{3}} - T^{\frac{1}{3}} T^{\frac{2}{3}}) / (d^{\frac{1}{3}} - d^{\frac{1}{3}})$	$a = ((S^2 - S + 2) / (S - 2)) T^{\frac{2}{3}} R^{\frac{2}{3}} / S^{\frac{2}{3}} P^{\frac{2}{3}}$	$a = 5.835 \times 10^8 T^{\frac{2}{3}} V^{\frac{2}{3}} / S$ (Approximate)	$a = M(\lambda^{\frac{1}{3}} - d^{\frac{1}{3}}) / (d^{\frac{1}{3}} - d^{\frac{1}{3}})$	$a = 1.259 \times 10^{11} (M \times V a)^{\frac{1}{3}}$



TABLE 9—(Continued)

Hexane	28.59 (8.2°)	27.72 (68.1°)	27.32	28.33	28.96	28.33	28.38	28.14	27.71
Octane	44.72	41.86 (15.5°)	40.01 (Diisobutyl)	41.98	—	41.98	42.10	<45.75	40.17
CCl <sub>4</sub>	24.01	22.63 (75.2°)	22.60 (75.2°)	24.53	24.54 (20°)	24.53	24.34	<22.02	22.94
Ether	20.33	20.34 (20°)	19.12	20.21	20.34 (20°)	20.21	20.17	19.45	20.46
Methyl acetate	17.43	—	15.90	17.01	17.55 (10°)	17.01	17.06	17.72	17.42

and  $\mu$ , the heat rendered latent by an increase in the intramolecular energy, is 14.80 cal. While the figure 14.80 is undoubtedly a little too low it appears that approximately one-fifth of the total internal latent heat goes within the molecules at the fraction  $0.313T_c$ . Furthermore, the internal intramolecular latent heat does not increase much, if at all, at temperatures lower than this. The theoretical latent heat at absolute zero was calculated by Mills' formula, and  $d_o$  was taken as  $Sd_c$ .

### 5. Computation of "a" from the Number of Valences and Molecular Weight

Finally I have calculated "a" for a gram mol by the formula:<sup>1</sup>

$$(34) \quad a = C(M \times \text{Val})^{2/3}.$$

C is taken arbitrarily as equal to  $1.259 \times 10^{11}$ . M is the molecular weight, and Val the numbers of valences per molecule.

The values computed in these different ways are given in Table 8. For the surface tension computations I have taken the data from Ramsay and Shields, Ramsay and Ashton and Renard and Guye. In all cases when computing "a" by the last formula the valence of carbon has been taken as 4; oxygen as 2, except in oxygen gas, where it is unity; nitrogen as 3, except in nitrogen gas which has been taken as monovalent; and hydrogen as 1. The critical data of oxygen are accurately determined; for hydrogen, the critical density being uncertain, I have assumed S to be 3.4, the same as oxygen; in computing nitrogen, the critical density being uncertain, I took S as 3.5 and the density 0.33, which is between the values given by Sarrau and Hautefeuille and Cailletet. For all substances included in his list Young's critical data have been used. The critical temperatures and pressures of the other substances have been taken from the Landolt-Meyerhoffer tables. Where the critical density was unknown I have been unable to compute by formulas which involve that factor. In other cases the surface-tension data or the vapor densities could not be found. Hence there are many gaps in the table.

<sup>1</sup> Mathews: Jour. Phys. Chem., 17, 181 (1913).



It will be seen by an inspection of Tables 8 and 9 that all the formulas, *i. e.*, those from the latent heat; from van der Waals' equation, assuming that  $b_c$  is  $2V_c/S$ ; from the surface tension; from Young's formula and that involving the molecular weight and number of valences, give practically the same result. The confirmation of the values from the surface tension and van der Waals' equation by the computation from the latent heat close to the critical temperature is, I think, conclusive evidence that these results are correct, within the limits of error of the data from which they are computed. The internal pressures of such liquids as benzene and ether are, therefore, about 14 per cent. higher than have been calculated by van der Waals' formula:  $a = 27T_c^2/64 \times 273^2P_c$ . As a result the co-volume, or the volume of the molecules, the value " $b$ ," must be taken larger both in the liquid and vapor than has been customary.

The values for " $a$ " and  $M^2K$  given in my earlier papers should be multiplied by 1.085 approximately to bring them to these new and correct values.

The internal pressures at zero degrees centigrade computed by Lewis, by the method of van der Waals and by formula (7) compare as follows (Table 10):

TABLE 10—INTERNAL PRESSURES IN ATMOSPHERES AT ZERO DEGREES

	Lewis	v. d. Waals	Formula (7) Surface tension
Ethyl acetate	2466	2261	2507
Ether	1932	1723	1986
Carbon tetrachloride	2518	2205	2637
Carbon bisulfide	2917	3363	3937
Benzene	2639	2494	2946
Toluene	2847	2228	2534
<i>m</i> -Xylene	2815	2068 (10°)	2286 (10°)

The values obtained from the surface tension and from the latent heat of expansion as computed by Lewis, agree pretty well except in the case of carbon bisulphide; they are widely

different from those computed by Walden from Stefan's conclusion, Walden's values being in fact about two-thirds of my values. The figures given for the internal pressures of liquids by Walden, Davies, and Traube are certainly far too low and are erroneous.

### Summary

The internal pressures of liquids, or rather the value "*a*" of van der Waals' equation, has been computed from the surface tension, assuming that the depth of the surface layer is  $(T_c/(T_c - T))^{2/3}$  molecular diameters; from the law of Eötvös and T. Young; from van der Waals' equation, assuming that  $b_c$  is always  $2V_c/S$ ,  $S$  being equal to  $RT_c/V_cP_c$ ; from the internal latent heat of vaporization close to the critical temperature, and from the molecular weight and the number of valences. All of these methods give practically the same results. The values of "*a*" thus computed are constant in the case of pentane and ether over a wide range of temperature, indicating that barring association "*a*" is constant; the values are uniformly higher than those computed by others with the exception of some computed recently by Lewis from the latent heat of expansion of liquids. The values recently given by Traube, Walden and Davies are too low and incorrect in other ways. The results confirm my conclusion that the molecular cohesion is a function of the molecular weight and the number of valences in the molecule. The formula:  $a = 27T_c^2/64 \times 273^2P_c$  gives values about 14 per cent. too low for ordinary substances and very much too low for simple diatomic gases. It should be replaced by the formula  $a = (S^2 - S + 2)T_c^2R^2/(S^2(S - 2)P_c)$ . These results show, also, that Stefan's conclusion that half the work in vaporization is done in moving a particle into the surface is incorrect.<sup>1</sup>

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<sup>1</sup> APPENDIX.—By combining (23) and (26) we have the following formulas for calculating  $S$  and  $d_c$ .  $M$  is the molecular weight:

$$(35) \quad S^2 = (d_g - d_v) \frac{R^{4/3}}{T_c^{1/3}} / (T_c - T)^{1/3} MP_c$$

$$(36) \quad d_c^2 = MP_c(d_g - d_v) / RT_c^{2/3} (T_c - T)^{1/3}.$$





## THE QUANTITY OF RESIDUAL VALENCE POSSESSED BY VARIOUS MOLECULES

BY A. P. MATHEWS

All, or nearly all molecules possess some power of combining with molecules of the same or different kinds. This combining power is called the residual valence, or affinity, of the molecule. Thus ammonia,  $\text{NH}_3$ , will unite with water or acids, a molecule of hemoglobin with oxygen, glucose and probably all salts with water when they dissolve in it, and other examples of this power of molecular union might be given.

The importance of residual valence to the molecule is generally recognized. It is probable that, with the possible exception of ionic reactions, these molecular unions precede, and are a necessary condition for, most chemical interactions between molecules; for molecules do not seem to affect each other by simple contact, but only when they are united into a new molecule by chemical bonds. It would seem that it is only when they are thus united that the atoms of two molecules are able to interchange and undergo those rearrangements resulting in the birth of new molecular species.

The importance of residual valence makes it desirable to know how much of it is possessed by each species of molecule. Since it is possible that this amount may not always be the same even for the same species of molecule, a method must be used in determining the average amount which will examine the molecular system without disturbing it. For example not all the molecules of carbon dioxide may be in a condition to unite with water at the same instant. Many facts indicate that of all the molecules of oxygen in the air only a few, at any instant of time, are in a condition to unite with oxidizable substances. The number possessing residual valence is small. This changing molecular condition seems analogous to, and is very possibly essentially identical with, the varying condition of atoms of radium which only occasionally become



radioactive and decompose. It is not impossible, on the electronic theory of valence, to ascribe the acquiring of additional or residual valence by the atoms of molecules to internal rearrangements of the electrons within the atoms, similar to that rearrangement which, in radioactive substances, leads to an explosion of the atom.

The method I have used to determine the quantity of residual valence is a physical one, based on the cohesion of molecules. While the method is not very accurate at present, owing to several doubtful points in the calculations, it probably places the molecules in the order in which they occur when arranged according to the amount of average residual valence they possess; and it will become more precise as the critical data are more accurately known and the cohesion or internal pressure more accurately determined. The method is as follows: The internal or cohesive pressure of a fluid is represented by the value  $a/V^2$  of van der Waals' equation. In this expression  $V^2$  represents how the cohesive attraction varies with the distance; and " $a$ " is the "mass" factor of the attraction. Now " $a$ " includes the factor  $N^2$ ,  $N$  being the number of molecules in the volume  $V$ , and if " $a$ " is divided by  $N^2$  then the quotient represents the "mass" factor of the cohesive attraction of two molecules and it may be written  $M^2K$  to correspond with the mass factor of the gravitational attraction,  $m^2k$ , in which " $m$ " is the gravitational mass and " $k$ " the gravitational constant. The relationship was found<sup>1</sup> that  $M^2K$  is proportional to the two-thirds power of the product of the molecular weight by the number of valences in the molecule, or  $M^2K = C(Wt. \times Val.)^{2/3}$ . How accurately this relationship holds is shown in Table I in which the value of " $a$ " which is of course proportional to  $M^2K$ , and is the mean value computed from various formulas, is compared with the value of " $a$ " computed from the molecular weight and the number of valences. The method of computing " $a$ " was given in another paper.<sup>2</sup>

<sup>1</sup> Mathews: Jour. Phys. Chem., 17, 181 (1913).

<sup>2</sup> Mathews: Ibid., 17, 603 (1913).

TABLE I

Comparison of "*a*" computed from the critical data, etc., with "*a*" computed from the molecular weight and valence. The figures represent dynes for gram mol amounts, multiplied by  $10^{-12}$

Substance	Mean value of " <i>a</i> "	$a = C(\text{Wt.} \times \text{Val.})^{2/3}$ ( $C = 1.259 \times 10^{11}$ )	Difference Actual	Difference Percent
H <sub>2</sub>	0.311	0.317	-0.006	-1.89
N <sub>2</sub>	1.836	1.842 (Val. = 2)	-0.006	-0.31
O <sub>2</sub>	1.984	2.014 (Val. = 2)	-0.030	-1.5
<i>n</i> -Pentane	22.07	21.96	+0.11	+0.5
<i>i</i> -Pentane	21.41	21.96	-0.55	-2.2
<i>n</i> -Hexane	28.10	27.71	+0.39	+1.38
Heptane	34.82	33.80	+1.02	+3.06
Octane	41.94	40.17	-1.77	+4.4
Diisobutyl	40.01	40.17	-0.16	-0.4
Ether	19.94	20.46	-0.72	-0.32
Benzene	21.95	22.19	-0.24	-0.11
Chlor-benzene	23.26	22.94	+0.32	+0.14
Toluene	28.12	27.97	+0.15	+0.50
Metaxylene	34.08	34.06	-0.02	+0.06
Methyl formate	12.04	12.25	-0.21	-1.7
Methyl acetate	17.12	17.42	-0.30	-1.1
Methyl propionate	22.49	22.96	-0.47	-2.0
Ethyl acetate	22.84	22.96	-0.12	-0.5
Propyl formate	23.13	22.96	+0.17	+0.72
Methyl butyrate	29.53	28.84	+0.69	+2.4
Methyl iso-butyrate	28.27	28.84	-0.60	-2.1
Propyl acetate	28.95	28.84	+0.08	+0.3
Ethyl propionate	28.92	28.84	+0.05	+0.2
SnCl <sub>4</sub>	32.58	32.58 (Val. = 16)	±0.00	±0.00

From the equation  $a = C(\text{Wt.} \times \text{Val.})^{2/3}$ , if  $M^2K$ , or "*a*," can be determined, and if *C* and the molecular weight are known, the total number of valences in the molecule can be calculated. If from this total number of valences there be subtracted the number which is known to exist in the molecule on the basis that hydrogen is univalent, oxygen bivalent, carbon quadrivalent and so on, the remainder may be supposed to constitute the *average* amount, or number, of extra or residual valences which the molecule possesses. It is this number which has been determined in this paper.



Granting that this number really represents the residual valence, the accuracy with which it can be determined will depend on the accuracy with which  $M^2K$ ,  $C$ , the molecular weight and the number of valences reaching between the atoms can be determined. The molecular weight may be assumed to be normal for non-associating substances at the critical temperature; and we have to assume that the number of valences between the atoms are those which chemists usually assign to these elements. The determination of the constant  $C$ , however, is more difficult. It could be determined empirically if  $M^2K$  was known accurately for any substance of which the valence is fixed and certain. Hydrogen is the only element with an unchanging valence, but unfortunately the critical pressure of hydrogen is uncertain. Moreover, it cannot be assumed that the valence of hydrogen is exactly unity. There are several indications that a molecule of hydrogen has some residual valence although it is certainly small in amount. One such indication is the solubility of hydrogen in water. At the same pressure and temperature more molecules of hydrogen dissolve in water than of helium, and hydrogen is half as soluble as nitrogen which almost certainly has residual valence. If solubility involves residual valence, as it may, this means that hydrogen would have some residual valence. Its solubility in platinum may be interpreted in the same sense. The catalytic reducing action of platinum, nickel or other metals or metallic oxides in a hydrogen atmosphere is interpreted by Sabatier<sup>1</sup> to mean that a chemical union of the reacting substances occurs. Armstrong,<sup>2</sup> too, has expressed the opinion that hydrogen has a small amount of residual valence. For these reasons we cannot accurately determine  $C$  from hydrogen. Nevertheless I have calculated  $M^2K$  and  $C$  for hydrogen to show what the value of  $C$  would be if hydrogen were univalent.

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<sup>1</sup> Sabatier: Nobel Prize Address, p. 9, *et seq.*; Les Prix Nobel en 1912; La Méthode d'Hydrogénation directe par Catalyse.

<sup>2</sup> Armstrong: "Valency," Encyclopaedia Britannica, 11th Ed., 27, 848 (1911).

Using the critical data of  $T_c = 32.3$  and  $P_c$ , 13 atmospheres as found by Olszewski; and  $d_c$  as 0.033 as determined by Dewar, the critical coefficient  $S$ , where  $S = RT_c/P_c V_c$ , is equal to 2.860. This value of  $S$  is, however, much lower than that of any other substance. Thus for  $O_2$ ,  $S$  is 3.5; for  $N_2$ , 3.6; for  $N_2O$ , 3.4 and even for helium it is 3.13 according to Onnes. It is probable then that 2.86 is too low. The critical temperature and pressure have recently been determined by Buller<sup>1</sup> who finds  $T_c = 31.95$ ;  $P_c = 11$ . With these values and  $d_c = 0.033$   $S$  computes 3.903, which is higher even than such complex substances as octane and is clearly too high. If  $S$  is calculated by my formula which gives a result within 1-2 percent in most cases, namely,  $S^2 = R(d_1 - d_v)T_c^{4/3}/(T_c - T)^{1/3}MP_c$ , using Buller's values for  $P_c$  and  $T_c$  and taking  $d_1$  as that of hydrogen at the melting point [ $-258.9^\circ$  (Travers)] as 0.086 (Dewar) and disregarding the value of  $d_v$  at that temperature then  $S$  is computed as 3.517. If  $P_c$  were 11.5 atmospheres, and the uncertainty is at least half an atmosphere,  $S$  would be 3.365. I believe  $S$  of hydrogen may be taken as approximately that of oxygen or as 3.4. If  $S$  is assumed to be 3.4 then with  $P_c$  11 and  $d_c$  0.033,  $a = (S^2 - S + 2)/(S - 2) P_c V_c^2 = 0.301 \times 10^{12}$ . Dividing this by  $N^2$  where  $N = 6.062 \times 10^{23}$ ,  $M^2K$  for hydrogen would be  $8.191 \times 10^{-37}$  from which  $C$  is found to be  $3.23 \times 10^{-37}$ . If, however,  $S = 3.4$  and  $P_c$  13, then " $a$ " would be  $0.317 \times 10^{12}$ ,  $M^2K$ ,  $8.626 \times 10^{-37}$  and  $C$  would be  $3.45 \times 10^{-37}$ . Since from Buller's results it is probable that  $P_c$  should be lower than 13 atmospheres, the value for  $M^2K$  is probably not far from correct so that  $C$  should be very nearly  $3.23 \times 10^{-37}$ .

Another simple substance from which a calculation of  $C$  might be made is methane, since the amount of its residual valence is certainly small and the total number of valences per molecule is very nearly 8. Unfortunately the critical density of methane is unknown and  $T_c$  and  $P_c$  have not been recently determined. However, if  $T_c$  is 191.2 and  $P_c$  is 54.9

<sup>1</sup> Buller: Phys. Zeit., 14, 860-2 (1913).



(Olszewski) and if  $S$  be calculated by the formula already given using the value of the density of the liquid at  $-164^{\circ}$  as 0.466 and disregarding the vapor density,  $S$  computes to be 3.318 which is probably not far wrong. From this " $a$ " is found by the formula given above to be  $3.025 \times 10^{12}$ ,  $M^2K$  is  $8.232 \times 10^{-36}$  and  $C$  is found to be  $3.24 \times 10^{-37}$  which is almost the same value as that computed from hydrogen.

The critical data of oxygen are known, but the calculation shows clearly that oxygen is monovalent in the elemental form, there being but two valences in the molecule. This unexpected conclusion makes it impossible to use oxygen for the determination of  $C$  until it can be shown from independent sources that oxygen in the elemental form is really monovalent. There is some residual valence also. But if the residual valence be disregarded and two valences only be postulated in the molecule, the value of  $C$  would be  $3.43 \times 10^{-37}$ .

The mean value of  $C$  determined from all the substances in Table VIII in my former paper was  $3.45 \times 10^{-37}$ , if Millikan's value of the number of molecules in a gram mol., namely  $6.062 \times 10^{23}$ , is used in the computation. Since in this calculation of  $C$  the molecules were not supposed to have residual valence, it is clear that an allowance for the presence of this valence would have the effect of lowering  $C$ , so that its true value must be somewhat less than  $3.40 \times 10^{-37}$ .

A way in which  $C$  can be independently determined was suggested by the relation between cohesion and gravitation.<sup>1</sup> In the formula  $M^2K = C(\text{Wt.} \times \text{Val.})^{2/3}$  it is evident that  $M^2K$  is proportional to the  $2/3$ ds power of the gravitational mass of a molecule and when weight and valence are unity  $M^2K = C$ . It occurred to me that under these circumstances  $C$  might very possibly be nothing else than the factor  $(m^2k)^{2/3}$  of a molecule of unity molecular weight. In this case " $m$ " is the gravitational mass of such a molecule and " $k$ " the gravitational constant. A computation of  $(m^2k)^{2/3}$  using Millikan's recent determination of the number of mole-

<sup>1</sup> Mathews: Jour. chim. phys., 1914.

cules in a gram mol, gave the result  $(m^2k)^{2/3} = 3.20 \times 10^{-37}$  which is very close to the figures already obtained from methane and hydrogen, and somewhat less, as it ought to be, than the mean value of  $3.45 \times 10^{-37}$  which was obtained when residual valence was disregarded. In view of these facts I believe we may assume that  $3.201 \times 10^{-37}$  is the real value of C, although the theoretical basis of this relationship is still lacking, and proceed with the calculation of the total valence of a molecule on that assumption.

The value of  $M^2K$  is less satisfactory. It is here that the main uncertainty of the calculation lies. As I have already discussed the methods of calculating this value in my paper on the internal pressures of liquids I will not go into the question at this time further than to point out two or three considerations bearing on the probable accuracy of those figures. In all the formulas for " $a$ " which have so far been proposed certain assumptions have been made. The one ordinarily made in van der Waals' method of computing " $a$ " is that  $b_c = V_c/3$ . In the various methods I have proposed for the computation quite different assumptions have been made in the different formulas, but nevertheless these formulas have all given results which are not widely different if the uncertainty of some of the experimental data are considered. Nevertheless, the formulas do not always give exactly the same values as they should if all the assumptions and data were rigorously correct. The computation of the cohesion from the latent heat of vaporization should give a correct result since the assumptions made here are less radical than in any of the other methods. Now this method generally gives a value for " $a$ " lower, in some cases 5 percent lower, than that computed from the critical data. But I have not been able to attach more importance to this deviation for the reason that the computation must be made close to the critical temperature, within a fraction of a degree of it, and a very slight error in the difference of the vapor and liquid densities would make a very large error in " $a$ ." That the formulas proposed for " $a$ " are possibly not entirely accurate may be shown also



by the following circumstance: From the formula  $a = ((S^2 - S + 2)/(S - 2))P_c V_c^2$  and the formula  $a = M((S^2 - S + 2)/(S - 2))RT_c^{3/2}(T_c - T)^{1/2}/(d_1 - d_v)$  we have  $V_c^2 = MR(T_c - T)^{1/2}T_c^{3/2}/(d_1 - d_v)P_c$ . If now we compute  $d_c$  of oxygen by this formula from the densities of liquid and vapor oxygen found by Mathias and Onnes, we find indeed a constant value for  $d_c$ , but a density nearly 3 percent higher than that computed by the rectilinear diameter law, as follows:

TABLE II

Computation of the critical density of oxygen from the densities at different temperatures,  $t$

$t$	$d_c$
—118.8°	0.4413
—120.4	0.4428
—140.2	0.4437
—154.51	0.4427

$d_c$  computed by Mathias and Onnes by the rectilinear diameter law was 0.4299.

The deviation with pentane was in the opposite direction.  $d_c$  was found by S. Young to be 0.2323. If it is calculated by the foregoing method from Young's density figures we have

$t$	$d_c$
0°	0.2235
160	0.2265

In this case the result was about 3 percent too low.

With octane the computed and found values agree very well, as follows, using Young's density figures at various temperatures.

$t$	$d_c$
0°	0.2319
60	0.2324
120	0.2336
160	0.2348
190	0.2352
230	0.2372

The density values show a tendency to advance. The mean value of about 0.2330 is very close to that determined by Young of 0.2327.

The critical density calculated for various other substances from the liquid and vapor densities resulted as follows when compared with the found values:

Substance	Temperature and density from which calculation made	$d_c$ Calculated	$d_c$ Found
Benzene	$0^\circ d_1 = 0.90006; d_v = 0.00012$	0.3019	0.3045
Fl-benzene	$0^\circ d_1 = 1.04653$	0.3495	0.3541
Br-benzene	$0^\circ d_1 = 1.52182$	0.4804	0.4853
CO <sub>2</sub>	$0^\circ d_1 = 0.914; d_v = 0.096$	0.4749	0.46
CCl <sub>4</sub>	$0^\circ d_1 = 1.63255$	0.5569	0.5576
Ethyl ether	$0^\circ d_1 = 0.7362; d_v = 0.000827$	0.2605	0.2625
	$60^\circ d_1 = 0.66580; d_v = 0.006771$	0.2622	

The foregoing figures show that the formula used for the calculation of  $d_c$ , which was derived from two of the formulas used in the calculation of " $a$ ," gives results which agree generally within 1 percent of the values of the critical density determined by experiment, but in some cases there is a deviation of about 3 percent. We may, I think estimate the uncertainty in the value of " $a$ " and hence of  $M^2K$ , as not more than 2-3 percent.

The formula which I have chosen for the calculation of the value of " $a$ " is that which is based on the assumption that the value of  $b_c$  is  $2V_c/S$ . This formula is:  $a = ((S^2 - S + 2)/(S - 2))P_cV_c^2$ . This equation involves only the critical data and may be applied to the largest number of substances.

While the calculation of the total valence of the molecules is thus subject to these uncertainties, it is probable that the substances are arranged in their proper order of the amount of residual valence and that the error in the total valence of the molecule is not more than 5 percent at the outside. The results are given in Table III. The values of " $a$ " are taken from column 4 of Table VIII of my paper<sup>1</sup> on the internal pressures of liquids.

<sup>1</sup> Mathews: *Loc. cit.*, p. 622.



TABLE III  
Amount of residual valence

I Substance	II Formula	III Theoretical No. of val- ences	IV Total No. of valences by formula $a = C^1(\text{Wt.} \times \text{Val.})^{2/3}$	V Resid- ual valence
Hydrogen	H <sub>2</sub>	2	2.195	0.195
Oxygen	O <sub>2</sub>	2	2.195	0.195
Nitrogen	N <sub>2</sub>	2	2.197	0.197
Nitrous oxide	N <sub>2</sub> O	4(?)	6.765	2.765
Ethylene	C <sub>2</sub> H <sub>4</sub>	12	12.904	0.904
<i>n</i> -Pentane	C <sub>5</sub> H <sub>12</sub>	32	36.59	4.59
<i>i</i> -Pentane	C <sub>5</sub> H <sub>12</sub>	32	35.95	3.95
<i>n</i> -Hexane	C <sub>6</sub> H <sub>14</sub>	38	43.42	5.42
Diisopropyl	C <sub>6</sub> H <sub>14</sub>	38	42.41	4.41
Heptane	C <sub>7</sub> H <sub>16</sub>	44	50.89	6.89
<i>n</i> -Octane	C <sub>8</sub> H <sub>18</sub>	50	59.08	9.08
Diisobutyl	C <sub>8</sub> H <sub>18</sub>	50	56.30	6.30
Ether	C <sub>4</sub> H <sub>10</sub> O	28	30.45	2.45
Carbon tetra-chloride	CCl <sub>4</sub>	16 (Cl = 3)	20.28	4.28
Benzene	C <sub>6</sub> H <sub>6</sub>	30	33.70	3.70
Chlor-benzene	C <sub>6</sub> H <sub>5</sub> Cl	32	36.40	4.40
Methyl formate	C <sub>2</sub> H <sub>4</sub> O <sub>2</sub>	16	18.12	2.12
Ethyl formate	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	22	24.39	2.39
Methyl acetate	C <sub>3</sub> H <sub>6</sub> O <sub>2</sub>	22	23.52	1.52
Methyl propionate	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	28	29.38	1.38
Ethyl acetate	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	28	29.20	1.20
Propyl formate	C <sub>4</sub> H <sub>8</sub> O <sub>2</sub>	28	31.16	3.16
Methyl butyrate	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	34	36.11	2.11
Methyl iso-butyrate	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	34	35.30	1.30
Propyl acetate	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	34	36.05	2.05
Ethyl propionate	C <sub>5</sub> H <sub>10</sub> O <sub>2</sub>	34	35.80	1.80
Stannic chloride	SnCl <sub>4</sub>	16 (Cl = 3)	17.68	1.68
Carbon bisulfide	CS <sub>2</sub>	16 (S = 6)	15.94*	—
Methane	CH <sub>4</sub>	8	8.15	0.15

\* "a" computed from the surface tension.

These figures speak for themselves, but a word of comment may be made on some of them. It seems probable from the greater solubility in water of oxygen and nitrogen than hydrogen, that the average residual valence of a molecule

$$^1 C = 3.20 \times 10^{-37} \times N^2.$$

of hydrogen gas is somewhat lower than the figures indicate. In the series methane, ethylene, pentane, hexane, heptane and octane the residual valence is, respectively, 0.15, 0.9, 4.6, 5.42, 6.89, 9.05. In other words, the residual valence increases with the number of carbon atoms as Armstrong has already inferred it should do. If 0.15 be considered as the average amount of residual valence of a carbon atom when united with hydrogen and if the amount of residual valence increases proportional to the square of the number of carbon atoms, we should have  $R. Val. = n^2 0.15$ ,  $n$  being the number of carbon atoms. By this formula the number of residual valences in the series mentioned should be, respectively, 0.6 for ethylene, 3.75 for pentane, 5.40 for hexane, 7.35 for heptane, and 9.60 for octane. These values are not very different from those actually found. This relationship does not hold for the esters.

Another fact may be noticed, namely, that the differences between the total number of valences in the various groups of esters is very nearly the theoretical number. Thus between methyl formate and methyl acetate a difference of six valences is required. 5.40 was the difference found. If we take the average of the total number of valences found in the two esters of the formula  $C_3H_6O_2$  it is 23.95. This is 5.75 valences more than methyl formate has and is almost exactly six less than the next higher homologues of the formula  $C_4H_8O_2$ , of which the average number of valences found was 29.91. This in its turn is again 5.79 (required 6) valences less than the average of the next higher group of the formula  $C_5H_{10}O_2$ . Theoretically, there should be a difference of 32 valences between the molecule of hydrogen and a molecule of the formula  $C_5H_{10}O_2$ , whereas the method actually shows a difference of 33.62. It is certainly reasonable to suppose that this difference from the theoretical is to be ascribed to the larger amount of residual valence possessed on the average by a molecule of the ester as compared with hydrogen.

In the case of the chlorine compounds I have assumed that the valence of chlorine is three. The reason for this is



that I have not been able to find any chlorine compounds which show chlorine to have a lower valence than this. It might be assumed that these three valences were composed of one chief and two residual valences. In that case one should, of course, make the residual valence of chlor-benzene 6.40 instead of the value 4.40 which I have indicated. Another reason why I have not counted these two valences of chlorine as residual valences is that these chlorine compounds are non-associating compounds, or at any rate they associate very little. Hence the valences, sixteen in number, found in carbon tetra-chloride are probably not free residual valences, in the sense that they are valences in an active form but not saturated in the molecule, but they must be saturated in the molecule. On the other hand, the excess of 4.28 valences found above the calculated amount may or may not be in part saturated within the molecule.

It is evident then that the determination of the residual valence by this method of subtracting the theoretical number from the total number found is open to these serious sources of uncertainty. All that can be claimed for the method at this time is that it gives a method of calculating the total valences and thus estimating the residual valence, and that so far as indications go in the hydrocarbons and the esters the compounds are at least arranged in the order in which they would be placed, judging from their reactions, if arranged according to the amount of residual valence they possess. I hope that methods will be found to differentiate more clearly between the valences extending between the atoms and those additional valences extending outward from the atoms making the residual valence proper.

It is still too early to attempt to correlate the amount of residual valence with the solubility of compounds. It is at least possible that in solubility other factors than the number of valences come into play. The attraction between the molecules of solvent and solute may involve the factors which have been shown to influence cohesion, namely, molecular weight and number of valences, as well as the amount of

residual valence; it may also involve, of course, the amount of dissociation of the aggregates formed by cohesion or residual valence. It is probable, since the atomic unions are as a rule far more stable than the cohesive, the chemical attraction between the atoms being of an electro-static kind, that the union between solvent and solute due to the residual valence is of far more importance than that of a cohesional nature, just as the cohesional attraction is of vastly greater importance than the gravitational attraction; and there are not lacking indications that residual valence plays a very important part in solubility. I may mention in this connection the series helium to xenon already discussed elsewhere; the great dissolving power of associated as contrasted with non-associated liquids, shown by the solvent powers of water; the less association of associating substances when dissolved in associating solvents as compared with their state in non-associating solvents; the greater solubility of such gases as hydrogen sulphide, ammonia, sulphur dioxide which have greater residual valence than nitrogen, hydrogen and oxygen, and the fact that they are known to combine with water, and so on.

The residual valence is hardly ever found to be a whole number. The probable explanation of this is that the number found represents only the average amount of residual valence possessed by the molecules. It is probable that the residual valences open up in pairs, one positive and one negative, but that at any instant of time only a few molecules have them open, so that the average amount possessed by each molecule may appear to be a fraction.

### Summary

The amount of residual valence of a number of non-associating liquids and gases has been computed by subtracting from the total number of valences which the molecule possesses, as shown by its cohesion, the number which there is reason to believe extend between the atoms of the molecule. The difference is considered to be the residual valence.



The computation of the total number of valences in a molecule from the cohesion is made from van der Waals' factor " $a$ " by the formula :  $a = C(\text{Mol. Wt.} \times \text{Val. number})^{2/3}$ .  $a$  was computed by the formula already given, namely  $a = ((S^2 - S + 2)/(S - 2)) P_c V_c$ , in which  $S$  is the critical coefficient and  $P_c$  and  $V_c$  the critical pressure and volume. The constant  $C$  for a single pair of molecules was assumed to be equal to  $(m^2 k)^{2/3}$ , in which  $m$  is the gravitational mass of a molecule of unity molecular weight and  $k$  the gravitational constant. From Millikan's recent determination of the number,  $N$ , of molecules in a gram mol, the value of this constant was  $3.2015 \times 10^{-37}$  expressed in dynes. For a gram mol  $C$  is  $1.177 \times 10^{11}$ .

Owing to various uncertainties and assumptions in the calculations, this method of determination can be regarded only as of the nature of an approximation to the actual amount of residual valence of molecules.





## AN IMPORTANT CHEMICAL DIFFERENCE BETWEEN THE EGGS OF THE SEA URCHIN AND THOSE OF THE STAR-FISH.

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(Received for publication, April 21, 1913.)

The eggs of the sea urchin, *Arbacia punctulata*, differ markedly in their physiological properties from those of the star-fish, *Asterias forbesii*. The sea-urchin egg is remarkably stable, resistant to oxidation, has a very low rate of respiration and is not easily stimulated to artificial parthenogenesis; the star-fish egg, on the other hand, is, after maturation, very easily oxidized, has a rapid rate of respiration, forms sulphuretted hydrogen when mixed with sulphur, is easily destroyed by oxygen, easily liquefied by heat and is easily cytolysed by anesthetics. It is readily, even by shock, caused to develop parthenogenetically. Moreover after maturation a steady growth of the nucleus takes place, whereas in *Arbacia* the nucleus after maturation remains of a very small size.

Five or six years ago I found an important chemical difference between these eggs to be that cholesterol was lacking in the star-fish egg, but present in some quantity in that of the sea urchin. In view of the relation of cholesterol to hemolysis this observation offers a possible explanation of the great ease of cytolysis of the star-fish egg as compared with the sea-urchin.

The eggs were pressed from the ovary through cheese-cloth to remove the connective tissue, and the mass then extracted three times with a large amount of 95 per cent alcohol, boiling for one hour each time, and then once with boiling ether. The united extracts were evaporated on the water bath, the residue extracted with ether repeatedly, filtered from insoluble substances, and the ether poured into acetone. The fat and cholesterol remain in solution; the lecithin is precipitated. The acetone filtrate was evaporated to dryness, the oily residue saponified with alcoholic

sodium hydrate and, after the addition of sodium sulphate and some water, was shaken out with ether repeatedly. The ether was washed several times with sodium carbonate solution and evaporated to dryness. The residue was very small in amount, not crystalline; it looked like oleic acid. It gave no positive tests for cholesterol either by Salkowski's or the Liebermann-Burchard method. I have repeatedly sought for cholesterol in these eggs varying the procedure but I have never been able to find it. On one occasion when the ovaries were not ripe the fatty residue of the ether after repeated saponifications, both with alkali and acid, gave a very faint, transitory green such as cholesterol gives in the Liebermann test, and there may have been a very small amount of cholesterol present, but no crystals could be obtained. In view of the fact that the color reaction is probably not specific I am doubtful whether there was a trace of cholesterol present or not. It could not be positively identified. It may be mentioned that cholesterol in combination as in lanolin gives the Liebermann-Burchard reaction very strongly.

The same methods applied to the sea-urchin egg gave, as usual, a crystalline mass on evaporating the ether after saponification; the crystals looked like cholesterol and gave a typical reaction of Salkowski. I may say that the extract of the whole body of the star-fish contains cholesterol in abundance.

Another very interesting peculiarity of the star-fish egg is the character of its phosphatide. It resembles the jecorin described by Drechsel. A large quantity of eggs was extracted with hot alcohol and ether; the lecithin (?) precipitated from the ether solution in the usual way, redissolved in ether (not anhydrous) and reprecipitated with acetone and the process repeated until it dissolved quite clear in the ether and did not settle out a white substance when standing in the cold. This white substance coming out of the ether had a sweet taste, but had no reducing action on Fehling's solution either before or after heating with hydrochloric acid. The phosphatide thus prepared is more hygroscopic than lecithin from the brain or eggs. It makes unusually beautiful, regular myelin forms when shaken with water, and it seems to be toxic for sea-urchin eggs. It was probably not a pure substance. It contains a large amount of a reducing sugar which, calculated as glucose, amounts to 10.51 per cent by weight. The nature of this



sugar was not determined; an osazone was prepared; the fermentation test was indecisive. The lecithin itself does not reduce Fehling's solution but only after it has been heated with acid. I heated it for ten hours with 3.5 per cent HCl and determined the sugar by the reduction of Fehling's solution according to the method of Munson and Walker. This phosphatide also contains sulphuric acid in an ester form like Koch's sulphatide. It contained in a single analysis 1.19 per cent of sulphur in an oxidized form. This is in organic combination. The fatty acids are very largely oleic, or a similar acid, having an ether-soluble lead salt, The analysis of this impure phosphatide resulted as follows:

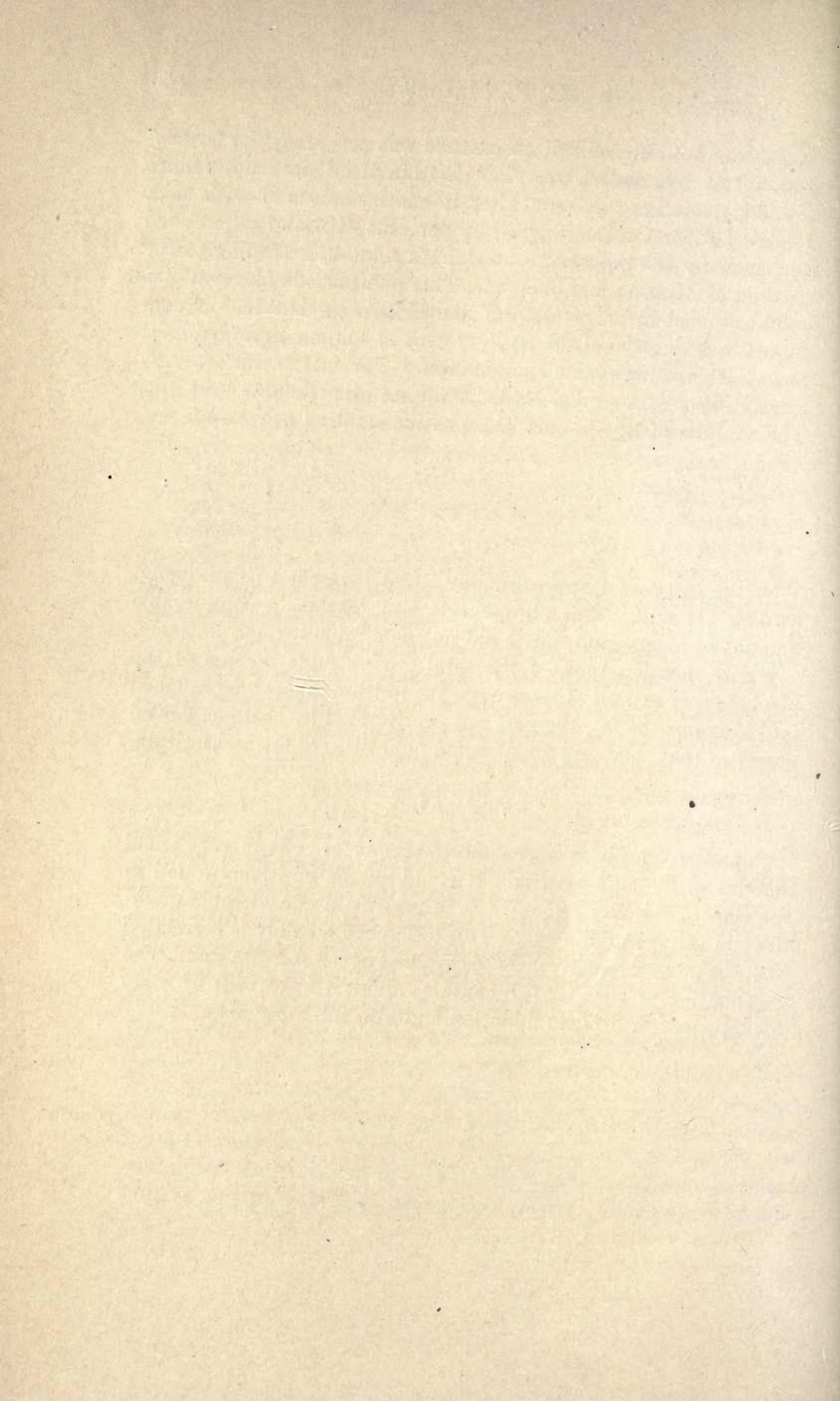
Glucose (?).....	10.51 per cent.
Fatty acids .....	46.16 per cent.
Phosphorus.....	3.57 per cent.
Sulphur .....	1.19 per cent.

Of the fatty acid approximately 71.35 per cent was recovered as oleic (?) acid. This phosphatide also contains a considerable amount of magnesium, but I did not determine it quantitatively.

I may mention that, of the total ether-soluble portion of the alcohol-ether extract of these eggs, the lecithin in one case weighed 0.6105 gram; the fat, the part not precipitated by acetone, 0.6055 gram; so that there are about equal quantities of fat and lecithin.

#### SUMMARY.

Cholesterol is either absent altogether or present in very small amount in the star-fish egg. It could not be positively found in the eggs of *Asterias forbesii*. It is present in considerable quantities in the sea-urchin egg. This difference possibly is correlated with the greater sensitiveness to cytolysis of the star-fish egg. The phosphatide of the star-fish contains about 10 per cent of a reducing sugar in firm combination and also sulphuric acid.





## CARBON DIOXIDE PRODUCTION FROM NERVE FIBRES WHEN RESTING AND WHEN STIMULATED; A CONTRIBUTION TO THE CHEMICAL BASIS OF IRRITABILITY.<sup>1</sup>

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### INTRODUCTION

THERE have been two theories of the nature of conduction — one upheld among others by Hermann, that it was a propagated chemical change; the other, at present the dominant view, that it is a propagated physical change.

In 1901 Professor Mathews suggested <sup>2</sup> that it was in the nature of a coagulative wave propagated along the fibre; this coagulation of the nerve colloids leading either directly or indirectly to the electrical disturbance accompanying the impulse. At the time, there was no evidence of chemical change in the nerve fibre, and its indefatigability seemed to point to an absence of metabolism. Certain facts were known, however, which were difficult to reconcile with this physical theory. Darwin had observed that in *Drosera*,<sup>3</sup> conduction occurred only if the protoplasm had oxygen; and Mathews <sup>4</sup> observed that salts would not stimulate a nerve, or, at any rate, their power of stimulation was much reduced if the nerve remained in the body for a time after death, or if the nerve were brought into the salt solution in an atmosphere of hydrogen. This clearly indicated a dependence of the irritability on oxygen.

<sup>1</sup> The preliminary report of these investigations was given in part in Biochemical section of Eighth International Congress for applied chemistry, September, 1912. See original communications, Eighth International Congress of applied chemistry, xxvi, p. 163. See also this Journal 1913, xxxi, p. xxii.

<sup>2</sup> Mathews: Century Magazine, 1902, pp. 783-792; Science, 1902, xl, p. 492.

<sup>3</sup> Insectivorous Plants, p. 57.

<sup>4</sup> Unpublished observations.

This fact lead to a search for evidence of the chemical nature of irritability and in a number of papers <sup>5</sup> it was clearly pointed out that the anaesthetics were probably acting directly in a chemical manner instead of indirectly, by affecting permeability, and that probably the anaesthetics acted by uniting with the protoplasm where O<sub>2</sub> usually took hold. This view was strengthened by the temperature coefficient of conduction, which is nearly that of a chemical reaction; by the importance of O<sub>2</sub> for artificial parthenogenesis; and by many other facts some of which have recently been collected by Haberlandt, Buitendijk and others.

Although it has been established by repeated demonstrations, that the nerve does not fatigue under ordinary conditions, as measured by the method used in muscular studies, yet Fröhlich <sup>6</sup> observed that the nerve undergoes certain changes by long activity. Gotch and Burch discovered <sup>7</sup> in 1889 that if two stimuli are successively set up within  $\frac{1}{500}$  of a second, only one negative variation is produced. This critical interval, or refractory period, is found to be altered by temperature changes, by drugs, asphyxiation, and anaesthetics.<sup>8</sup> Thus by prolonging the refractory period by partial anaesthesia, Fröhlich easily demonstrated that with a frequency of stimulation less than this normal refractory period, stimulation of the attached muscle no longer occurred. He interprets this as a phenomenon of fatigability of the nerve. Thöner's <sup>9</sup> observation seems to lead to a similar interpretation, for he found recently that fatigability is less effective when the refractory period is shortened by high temperature. There seems, then, to be fatigue in the nerve, but it cannot be measured by an ordinary scale.

After the complete failure of the chemical detection of CO<sub>2</sub> and

<sup>5</sup> A. P. MATHEWS: Biological bulletin, 1904-5, viii, p. 333; this Journal, 1904, xl, p. 455; *ibid.*, 1905, xiv, p. 203; Biological Studies by the pupils of William Sedgwick, 1906, p. 81; Journal of pharmacology and experimental therapeutics, 1911, ii, p. 234.

<sup>6</sup> FRÖHLICH: Zeitschrift für allgemeine Physiologie, 1903-4, iii, p. 445. *Ibid.*, p. 75.

<sup>7</sup> GOTCH and BURCH: Journal of physiology, 1899, xxiv, p. 410.

<sup>8</sup> See TAIT and GUNN, Quarterly journal of experimental physiology, 1908, i, p. 191; TAIT, *ibid.*, 1909, ii, p. 157.

<sup>9</sup> THÖNER: Zeitschrift für allgemeine Physiologie, 1908, viii, p. 530; *ibid.*, 1912, xiii, pp. 247, 267, 530.



acids in the excited nerve, Waller still believes that it must give off  $\text{CO}_2$  when stimulated. In 1896, he showed, with an electro-physiological method, that among other reagents,  $\text{CO}_2$ , in minute quantities, increased the excitability of the isolated nerve of the frog, and that the normal nerve, when excited, also increased its activity.<sup>10</sup> From this he ingeniously formed the hypothesis that every activity in the nerve fibre must be associated with  $\text{CO}_2$  production.

That there may be  $\text{CO}_2$  production in the nerve, but too small to be measured by ordinary methods, is shown by the following calculations: A frog (*Rana temporaria*) gives off 0.355 gram of  $\text{CO}_2$  per kilogram per hour at  $19 - 20^\circ \text{C}$ .<sup>11</sup> A small piece of the nerve fibre of the same animal, say 1 cm. in length, will weigh in the neighborhood of 10 milligrams. Now, if the mass of the nerve respires at the rate of the whole animal, it would give off about 0.0000007 grams of  $\text{CO}_2$  during ten minutes. This calculation at once suggested that the lack of positive evidence of metabolism in the nerve fibre was not at all conclusive that such metabolism did not occur, in view of the limitation of the methods for the estimation of  $\text{CO}_2$ . It was evidently necessary to devise methods for the detection of very minute quantities of  $\text{CO}_2$ . Thus at Professor Mathews' suggestion a new method for  $\text{CO}_2$  analysis was first devised, and then, under his direction, I have undertaken to go back once more to the question of  $\text{CO}_2$  production in the nerve fibre during the passage of a nerve impulse.

To study the nature of metabolism involved in a tissue, one should at least determine the oxygen consumption and the carbon dioxide production. Inasmuch, as the present problem, however, is concerned only with direct evidence for the existence of metabolism in the nerve fibre, I have attempted to measure  $\text{CO}_2$  production only, for it is true that the lack of oxygen consumption may not necessarily indicate the absence of chemical changes, while the production of  $\text{CO}_2$  will surely prove the presence of metabolism. Furthermore, as  $\text{CO}_2$  production is the only sure universal expression of the respiratory activity in anaerobic and aerobic plant and animal tissue in normal condition, the inquiry of  $\text{CO}_2$  production in an excited nerve will not only concern the problem of the nature of the nerve impulse

<sup>10</sup> WALLER: Croonian lecture, Philosophical transactions, London, 1896.

<sup>11</sup> Taken from Pott's figures. See figures in Table ix, p. 129.

itself, but may, also, aid in forming a fundamental conception of the tissue respiratory mechanism. In this way, if the protoplasmic irritability has a direct connection with the cellular respiration, then our idea of the general nature of the pharmacodynamics of many reagents on a living tissue may be essentially modified.

## METHODS AND MATERIALS

Two new apparatus were constructed which will detect  $\text{CO}_2$  in as small quantities as one ten-millionth of a gram and estimate it with quantitative accuracy. The detailed method has been described in a separate article.<sup>12</sup>

Preliminary experiments with these new apparatus showed that the sciatic nerves of dogs gave too large quantities of  $\text{CO}_2$  for my method so that I was compelled to use a smaller nerve of a cold-blooded animal for quantitative estimation. For exact measurements of  $\text{CO}_2$  production, I have used only two kinds of nerve, although I have used a large variety of nerves in qualitative experiments. For a non-medullated nerve fibre, Prof. G. H. Parker<sup>13</sup> was so kind as to suggest to me that I use the nerve trunk of the claws of the spider crab (*Labinia Caniliculata*) which is a bundle of mixed sensory and motor fibres. The frog, whose sciatic was used as a representative for medullated nerve, was exclusively *Rana pipiens*, obtained from Indiana.

As my apparatus in the present form cannot be used for a muscle nerve preparation nor for the normal nerve in situ, the use of an isolated nerve could not be avoided. Experimental factors thus introduced should be carefully considered before we interpret the observation as a normal metabolism. This serious objection, however, can be overlooked, as far as our fundamental question of different metabolic activities before and after a stimulation is concerned, for Waller<sup>14</sup> has demonstrated that the presence of excitability in an isolated nerve persists as long as nineteen hours provided that the electrical changes correctly represent the state of excitability. Although

<sup>12</sup> See pp. 137-145.

<sup>13</sup> For this and other suggestions, I am under great obligation to Dr. Parker.

<sup>14</sup> WALLER: 1896, Brain, xix, p. 53.



Herzen claims that under certain conditions of local narcosis the nerve fibre may give an action current without any muscular contraction (Wedenshi and Boruttau both deny this), and Ellinson<sup>15</sup> recently demonstrated by the use of cinchonamine hydrochloride the absence of negative variations without abolishing the excitability of the nerve, yet evidences are now abundant to indicate that the action current is a normal physiological phenomenon in uninjured tissue expressing the simultaneous activity resulting in a corresponding change in the peripheral organ.<sup>16</sup> These facts, therefore, must be taken as showing that as long as a negative variation remains, the nerve is probably excitable; and that the phenomena observed in the isolated nerve could be regarded as identical with that of a normal nerve as far as the passage of a nerve impulse in an isolated nerve fibre is concerned.

#### CO<sub>2</sub> PRODUCTION FROM RESTING NERVE

In this study of the metabolism of the resting nerve, particular care was taken to select those fibres which were free from nerve cells. The work of several investigators<sup>17</sup> seems to indicate that tissue oxidation is primarily concerned with the cell nucleus. Inasmuch as the respiration in the central nervous system is certain<sup>18</sup> and the blood supply to fibres is seemingly scanty, the notion persists among certain biologists that a nerve fibre should not respire since it has no nucleus. In order to test the correctness of such an idea, I have studied quantitatively the output of CO<sub>2</sub> from various lengths of nerve which are known to be free from nerve cells.<sup>19</sup> Here is the result:

<sup>15</sup> ELLINSON: *Journal of physiology*, 1911, xlii, p. i.

<sup>16</sup> For further details, see: GOTCH and HORSLEY: *Philosophical transactions of the Royal Society*, 1891, clxii, p. 514; BERNSTEIN: *Archiv für die gesammte Physiologie*, 1898, lxxiii, p. 376; REID and McDONALD: *Journal of physiology*, 1898-9, xxiii, p. 100; LEWANDOWSKY: *Archiv für die gesammte Physiologie*, 1898, lxxiii, p. 288; ALCOCK and SEEMANN, *ibid.*, 1905, cviii, p. 426.

<sup>17</sup> See SPITZER: *Archiv für die gesammte Physiologie*, 1897, lxvii, p. 615; M. NUSSBAUN: *Archiv für mikroskopische Anatomie*, 1886, xxvi, p. 485; R. S. LILLIE: *This Journal*, 1902, vii, p. 412.

<sup>18</sup> L. HILL: Quoted from *Hulliburton's Chemistry of nerve and muscle*, p. 79.

<sup>19</sup> In this connection, I wish to express my indebtedness to Prof. H. H. Donaldson for his kind advice.

**Non-Medullated Nerve Fibre.** — (The nerve of the spider crab, and apparatus 2 for the qualitative, and apparatus 1, for the quantitative, estimations were used.) When I place the nerve of a spider crab in the right chamber and no nerve in the left, and watch for the deposit of barium carbonate, the drop on the right will soon be coated with the white precipitate, but no precipitate whatever is visible with a lens in the left.  $\text{CO}_2$  is thus shown to be produced by this resting nerve. Now, by interchanging the nerve from the right to the left, no nerve being in the right, we can convince ourselves of the correctness of this conclusion, by eliminating any technical error which might produce the different results in different chambers. The rate at which the precipitate appears and the quantity of the precipitate, depends on the size of the nerve. In fact,  $\text{CO}_2$  production from the resting nerve of the spider crab is found to be proportional to its weight, other things being equal, and is constant: For 10 milligrams per ten minutes it gives  $6.7 \times 10^{-7}$  grams at  $15 - 16^\circ\text{C}$ .

The quantitative determination of this amount is made in the following manner:

The claws of the crab are carefully removed, and, by gently cracking them, the long fibre of the nerve trunk is easily isolated. After removing the last drops of the water by a filter paper, the nerve, with the aid of glass chop sticks, is carefully placed on the glass plate,<sup>20</sup> and quickly weighed. The glass plate with the nerve is now hung on the platinum hooks in the respiratory chamber A, and then the chamber sealed with mercury. The analytic chamber is now filled with mercury in the manner described elsewhere,<sup>21</sup> and then the apparatus is washed by  $\text{CO}_2$  free air as usual. The time when the barium hydroxide is introduced to the cup in chamber B is recorded, and the stop-cock between the two chambers is closed. When at the end of ten minutes the drop at cut F is perfectly clear, having not a single granule of the precipitate visible to a lens, thus insuring that the air is absolutely free from  $\text{CO}_2$  then a known portion of the gas from the respiratory chamber is introduced into the chamber below in which the clear drop of barium hydroxide has been exposed, and it is determined whether or not the amount of the gas taken contains

<sup>20</sup> The weight of this plate is known so that the weight of the nerve can be determined very quickly. See p. 120.

<sup>21</sup> See pp. 139.



- enough CO<sub>2</sub> to give the precipitate in ten minutes. If it does, a fresh nerve is prepared and a less volume of the gas is withdrawn; if it does not, a larger volume should be taken till the precipitate appears within ten minutes. (See footnote, page 140.)

In this way, by repeated experiments with several fresh nerves, a minimum volume of the gas for a known weight of the nerve which gives a precipitate is determined. This minimum volume should contain exactly a definite quantity of CO<sub>2</sub> — namely  $1.0 \times 10^{-7}$  gram.<sup>22</sup>

In this way, since we know the original volume of the respiratory chamber from which this minimum volume is withdrawn, and since we know the quantity of CO<sub>2</sub> contained in this volume, it is easily calculated, how much CO<sub>2</sub> is produced by the nerve during the known period. It should be understood that in determining the minimum volume of gas taken from the respiratory chamber, a series of experiments were conducted in order to calculate both the minimum volume which just gives the precipitate and the maximum volume which does not give the the precipitate for a known weight of the nerve for a known period of respiration. In the tables following, columns 8 and 9 refer to these volumes calculated from experiments.

Table I, gives the result for a non-medullated nerve.

**Medullated Nerve Fibre.** — For the quantitative estimation of CO<sub>2</sub> production from the medullated nerve I have taken a frog's sciatic, using apparatus 2. The results given in Table II, obtained by similiar methods, show that each ten milligrams of the frog's sciatic nerve gives off  $5.5 \times 10^{-7}$  grams for the first ten minutes.

A large quantity of nerves were tested and it was determined whether or not all resting nerves give off CO<sub>2</sub>. As a result, I found no exception in any of them. The following varieties of nerves were examined:

1. MOTOR NERVE: Occulo-motor nerve of the skate. (*Raia Ocallata*.)
2. SENSORY NERVE: Olfactory nerve of the same. (*Raia Ocallata*.)
3. MEDULLATED NERVE: Sciatic nerve of the dog, frog, turtle, mouse; optic nerve of the skate. (*Both Raia Ocallata and Raia Erinecia*.)
4. NON-MEDULLATED NERVES: Nerves of the spider crab; olfactory nerve of the skate. (*Raia Ocallata*.)
5. NERVE OF INVERTEBRATE: Spider crab's nerves.

<sup>22</sup> See p. 140.

TABLE I  
CO<sub>2</sub> PRODUCTION FROM RESTING NERVE OF SPIDER CRAB, LABINIA CANILICULATA (NON-MEDULLATED)

Column 1	2	3	4	5	6	7	8	9	10
Date	Temperature of room	Weight of nerve in milligrams	Stimulation	Duration of respiration in minutes	Amount of gas taken from respiratory chamber	Precipitation of BaCO <sub>3</sub> after ten minutes	No. of c.c. of gas which gives precipitate, calculated for 10 mg. nerve, ten minutes duration <sup>1</sup>	No. of c.c. of gas which <i>does not</i> give ppt. calculated for 10 mg. ten minutes <sup>1</sup>	Original volume of respiratory chamber
Oct. 13	15°8	40	no	30	2 c.c.	+	24 c.c.	....	9.5 c.c.
" "	18	20	"	30	1 c.c.	+	6 c.c.	....	"
Nov. 3	16.8	20	"	10	1 c.c.	+	2 c.c.	....	"
" "	"	20	"	10	.5 c.c.	+	....	1.0 c.c.	"
" 4	....	25	"	10	.5 c.c.	+	1.25 c.c.	....	"
" "	....	same nerve	"	10	.5 c.c.	+	....	1.25	"
" 5	....	16	"	10	1 c.c.	+	1.6 c.c.	....	"
" 6	15	20	"	10	.5 c.c.	+	....	1.0	"
" 7	14.8	16	"	16	.5 c.c.	+	.9 $\frac{1}{2}$ c.c.	....	"
" "	16	16	"	10	1 c.c.	+	1.6 c.c.	....	"
" "	16	16	"	10	1 c.c.	+	1.6 c.c.	....	"
" "	17.5	15	"	12	.55 c.c.	+	....	.99 c.c.	"
" 17	17	8	"	10	.5 c.c.	+	....	.4 c.c.	"
" "	17	12	"	10	.6 c.c.	-	....	.72 c.c.	"
" "	16	18	"	10	.6 c.c.	-	....	1.08 c.c.	"
" 8	14.8	8	"	10	1.5 c.c.	-	....	1.2 c.c.	"
" "	"	11	"	10	1 c.c.	-	....	1.1 c.c.	"
" "	16	12	"	10	.7 c.c.	-	....	.85 c.c.	"

<sup>1</sup> From these experiments, it is obvious that 1.25 c.c. out of respiratory chamber is minimum volume which gives the first precipitate. Since the original volume of respiratory chamber was 9.5 c.c. and 1.25 c.c. out of it contains the definite CO<sub>2</sub> to precipitate BaCO<sub>3</sub> which corresponds to  $1.0 \times 10^{-7}$  g., total CO<sub>2</sub> production from 10 mg. of this nerve for ten minutes is calculated as follows:

$$1.0 \times 10^{-7} \times \frac{9.5}{1.25} \text{ g.} = 6.7 \times 10^{-7} \text{ g. CO}_2 \text{ at } 15^\circ - 16^\circ$$

<sup>2</sup> This abnormal result is interesting, for this nerve was found hanging down from the glass plate, touching on the mercury at one end. Whether this high production of CO<sub>2</sub> was due to this or not was not determined.



TABLE II  
CO<sub>2</sub> PRODUCTION FROM RESTING SCIATIC NERVE OF FROG, RANA PIPIENS (MEDULLATED)

1	2	3	4	5	6	7	8	9	10
Date	Tempera- ture of room	Weight of nerve in milligrams	Stimulation	Duration of respiration	c.c. of gas taken from respiratory chamber	↓ of BaCO <sub>3</sub> after ten minutes	No. of c.c. which gives ↓, calculated for 10 mg. per ten minutes <sup>1</sup>	No. of c.c. which does not give ↓ calculated for 10 mg. ten minutes <sup>1</sup>	Original volume of respiratory chamber
March 26	19°	10	no	10 min.	1 c.c.	—	....	1 c.c.	15 c.c.
" "	....	same nerve	"	20 "	2 c.c.	+	4	....	"
" 27	21	11½	"	15 "	1.1 c.c.	—	....	2.47 c.c.	"
" "	21	11	"	10 "	1 c.c.	—	....	1.1 c.c.	"
" 28	21	6	"	10 "	2 c.c.	—	....	1.2 c.c.	"
" 31	20	13½	"	15 "	1 c.c.	—	....	2.02 c.c.	"
" "	20	14	"	15 "	1 c.c.	—	....	2.10 c.c.	"
April 1	19.5	9	"	15 "	2 c.c.	+	2.70	....	"
" "	20	9	"	15 "	2 c.c.	+	2.70	....	"
" "	19	16½	"	10 "	2 c.c.	+	3.30	....	"
" "	22	14	"	10 "	2 c.c.	+	2.8	....	"
" 2	21	11½	"	15 "	2 c.c.	+	2.65	....	"
" "	25	12	"	15 "	1.6 c.c.	+	2.4 <sup>2</sup>	....	"
" "	24	10½	"	20 "	1 c.c.	+	....	2.5 c.c.	"
" "	23	13	"	10 "	2.4 c.c.	+	2.6	....	"
" 3	13	20½	"	10 "	2 c.c.	—	....	....	"
" "	20	20½	"	10 "	1.2 c.c.	—	....	2.46 c.c.	"
" "	27	20	"	10 "	1.2 c.c.	—	2.40 <sup>2</sup>	2.46 c.c.	"
" "	29	26	"	10 "	1.2 c.c.	+	....	....	"
" "	25	25½	"	10 "	1 c.c.	—	....	2.6 c.c.	"
" "	18	22	"	11 "	1 c.c.	—	....	2.55 c.c.	"
" "			"		1 c.c.	—	....	2.2 c.c.	"

<sup>1</sup>By glancing at the columns 8 and 9 it is clear that 2.70 c.c. is the minimum volume, for 2.6 c.c. is maximum volume which does not give the precipitate. Since original volume of respiratory chamber is 15 c.c. we have

$$1.0 \times 10^{-7} \text{ g.} \times \frac{15}{2.7} = 5.5 \times 10^{-7} \text{ g. CO}_2 \text{ at } 19^\circ - 20^\circ$$

<sup>2</sup> Little high result in these cases is no doubt due to high temperature.

6. NERVE OF VERTEBRATE: Nerves of frog, dog, mouse, squiteague (*cynoscion Regalis*), and skate. (*Both Raia Ocallata and Raia Erinecia.*)
7. NERVE OF WARM-BLOODED ANIMALS: Those of dog, mouse and rabbits.
8. NERVE OF COLD-BLOODED ANIMALS: Frog, squiteague (*cynoscion Regalis*) and skate. (*Both Raia Ocallata and Raia Erinecia.*)

From this I have concluded that isolated nerves of all animals give off  $\text{CO}_2$ . It remains, now, to consider whether this  $\text{CO}_2$  is the product of normal respiratory activity or due to disintegration of the dead tissue.

#### IS THE $\text{CO}_2$ GIVEN OFF PRODUCED BY LIVING PROCESSES?

**Comparison of Dead and Living Nerves.** — In the first place, it was thought that if  $\text{CO}_2$  was due to normal metabolism of a living nerve, its production should be diminished when the nerve was killed. The following result (Table III) is self explanatory.

TABLE III

COMPARISON BETWEEN NORMAL AND KILLED (BY STEAM) NERVES OF SPIDER CRAB

1	2	3	4	5	6	7
Date	Temperature of room	Weight of nerve in mg.	Stimulation	c.c. of gas taken from respiratory chamber	Duration of respiration: minutes	Ppt. of $\text{Ba}(\text{CO}_3)$ after ten minutes
Nov. 4	13°	40 (killed)	no	.5	10	—
" "	..	40 (killed)	st'n	.5	10	—
" 5	..	16 (normal)	no	1.	10	+
" 6	15	16 (killed)	no	1.	12	—
" 7	16	16 (normal)	no	1.	10	+

#### Comparison of Anaesthetized and Non-Anaesthetized Nerves. —

It is naturally feared, however, that the killing experiment itself may not prove that  $\text{CO}_2$  production is necessarily due to the living mechanism, for high temperature may drive off  $\text{CO}_2$  produced already by the process of tissue disintegration, just as the  $\text{CO}_2$  diffused out from a wet thread saturated with the gas, the rate of diffusion being a function of temperature. Thus anaesthesia was tried, although we should



expect at the outset that if ether had no direct affect on the respiratory process, as some physiologists believe, then the negative results would not at all interfere with my contention. The fact is, however, that either an isolated nerve directly treated with ether vapor or urathane, or the nerve isolated from a deeply anaesthetized frog gave a much less quantity of  $\text{CO}_2$  than the normal nerve isolated from a normal frog whose heart has been cut away for a period of time equal to that of etherization. Anaesthetics, then, diminish  $\text{CO}_2$  production from an isolated nerve fibre. These experiments are being continued quantitatively.

#### $\text{CO}_2$ Production of Isolated Nerve at Successive Time Intervals.

— It was also thought that if  $\text{CO}_2$  production was due to bacterial decomposition, although it is highly improbable for such a fresh tissue, we may expect that either killing by steam or treating with

TABLE IV  
SHOWING DECREASED  $\text{CO}_2$  PRODUCTION BY LONG-STANDING (FROG'S SCIATIC)

1	2	3	4
Temperature of room	Time elapsed after isolation	Minimum c.c. necessary to give ↓ calculated for 10 mgs. 10 minutes	Total $\text{CO}_2$ produced from nerve of 10 mg. for 10 minutes
24°	immediately	2.7 c.c.	$5.5 \times 10^{-7}$ g. $\text{CO}_2$
25	1 hour	7.08 c.c.	$2.1 \times 10^{-7}$ g. $\text{CO}_2$
24	2 hours	10.8 c.c.	$1.4 \times 10^{-7}$ g. $\text{CO}_2$
24	5.5 hours	12.8 c.c.	$1.1 \times 10^{-7}$ g. $\text{CO}_2$
23.5	7 hours	15.3 c.c.	$.9 \times 10^{-7}$ g. $\text{CO}_2$
23.5	10.5 hours	21.0 c.c.	$.6 \times 10^{-7}$ g. $\text{CO}_2$
24	26 hours	9. c.c. <sup>1</sup>	$1.6 \times 10^{-7}$ g. $\text{CO}_2$
24	27.4 hours	1.8. c.c	$8.1 \times 10^{-7}$ g. $\text{CO}_2$

<sup>1</sup> The gradual increase at this point should be noted (after 26 hours, it is clear that bacterial decomposition sets in).

ether would check the  $\text{CO}_2$  production, and that the results observed above may not necessarily prove that  $\text{CO}_2$  production from the isolated nerve fibre is due to a respiratory process. Hence a number of the nerves were isolated from several frogs of the same size and sex, and

were left in Ringer's solution, and then the rate of the gas production is determined with the different nerves removed at successive intervals of time from the Ringer's solution for twenty-five hours. The interesting results given in Table IV not only show that  $\text{CO}_2$  from the fresh nerve is not due to bacterial decomposition, but it also indicates that when such abnormal decomposition sets in, the output of gas takes a sudden jump. This Table further shows that the vital process by which  $\text{CO}_2$  is produced gradually slows up as the tissue approaches death, indicating that the decrease of  $\text{CO}_2$  production is parallel to the decrease of irritability of the nerve.

**Increase of  $\text{CO}_2$  on Stimulation.** — The most convincing evidence of all that  $\text{CO}_2$  is formed by a vital process is the fact that a stimulated nerve gives off more  $\text{CO}_2$  (Part II) indicating the presence of normal metabolism in the living nerve which is accelerated when the nerve is stimulated. Thus we may safely conclude here that like any other tissue or organs, the nerve, too, respire whether it has a nucleus or not, and that the rate of  $\text{CO}_2$  production is proportionate to its weight, other things being equal.

#### $\text{CO}_2$ PRODUCTION FROM STIMULATED NERVE

We have now come to our main inquiry, namely, is there any chemical basis for irritability? Just what relation exists between nervous activity and chemical changes is the question that a biologist should consider before he attempts to build any conception of the real dynamics of living matter. For it is the phenomena of excitability in the nerve fibre that has stood so long in the path of understanding protoplasmic irritability in general. As for the brain, it is now established that certain chemical changes are involved during stimulation and that definite chemical changes are associated with pathological cases either in its chemical composition<sup>23</sup> or in the formation of abnormal metabolites.<sup>24</sup> Aside from the confused facts concerning histological changes in the ganglion cells of fatigued animals, Hill has observed, using Ehrlich's method of methylene blue

<sup>23</sup> KOCH and MANN: *Archiv of neurology and psychiatry*, 1909, iv, p. 44.

<sup>24</sup> DIXON: *Journal of physiology*, 1899-1900, xxv, p. 63; CROFTAN: *American journal of the medical sciences*, 1902, p. 150.



for the determination of the rate of oxidation, that a spot of cerebral surface, if stimulated, loses its blue color owing to the using up of the oxygen.<sup>25</sup> In case of the nerve fibre, however, we have already seen that no direct evidence has ever been presented to show any chemical changes connected with its activity, although there has been some indirect evidence. As considered before, the failure of the direct detection of CO<sub>2</sub> from the stimulated nerve must be due to the lack of a delicate method. Thus using the new method we have already demonstrated that a resting nerve gives off CO<sub>2</sub>, and will now attempt to prove that nerves give off more CO<sub>2</sub> when stimulated.<sup>26</sup>

**Electrical Stimulation of non-Medullated Nerve.** — Owing to the scope of delicacy of the new method, which is sensitive to as small a quantity as  $1.0 \times 10^{-7}$  gram (an amount corresponding to the CO<sub>2</sub> contained in  $\frac{1}{8}$  cc. of pure air), the utmost caution must be taken to prevent any complication which may result in formation or absorption of minute quantities of CO<sub>2</sub>. After I had found by experiment that there is no appreciable increase of CO<sub>2</sub> due to the direct electrical decomposition in the nerve when stimulated by a weak induction current and that several other forms of stimulation qualitatively confirmed the results obtained by the electrical stimulation, I have naturally employed the induction current as a stimulant in all my experiments on the quantitative estimation of CO<sub>2</sub> production from the stimulated nerve.<sup>27</sup>

As Table V shows, the stimulated non-medullated nerve fibre of the spider crab gives off  $16. \times 10^{-7}$  grams of CO<sub>2</sub> for 10 milligrams of

<sup>25</sup> HILL: *loc. cit.*

<sup>26</sup> Professor Carlson has very kindly called my attention to a recent publication from the Physiologisch Laboratorium der Utrechtsche Hoogeschool, in which Buijtendijk reports that certain head nerves of fishes take up more O<sub>2</sub> when electrically stimulated. He could not, however, find any increase of O<sub>2</sub> consumption in the sciatic of the frog. Also see: Koninklijk Akademie van Wetenschappen, Amsterdam, afd. xix, pp. 615-621.

Haberlandt also recently reports (*Archiv für Physiologie*, 1911, p. 419) that the resting nerve takes up of O<sub>2</sub>, 41.7 — 33.4 cmm. at 19° — 24° per gram per hour. When this nerve is excited, intake of O<sub>2</sub> is increased. Since the respiratory quotient of the stimulated nerve is equal to that of the resting, he concludes that when the nerve is excited, it must give off more CO<sub>2</sub>. He does not, however, indicate how much CO<sub>2</sub> is produced by stimulation.

<sup>27</sup> Use of non-polarizable electrodes was impossible for my apparatus, for the presence of foreign liquid in the chamber interferes with CO<sub>2</sub> estimation. As

nerve for ten minutes, while a fresh resting nerve gave only 6.7 by  $10^{-7}$  grams for the same units. The details of the methods are as follows:

The nerve of the claw of the spider crab is isolated as before. A comparative estimation was made first. Two pieces of the nerve of equal weights and length were placed separately on the two glass plates, each nerve being laid across the electrodes of the plate, in the manner shown in Figure 1. In this way either nerve can be stimulated at will. These glass plates are hung by their wires upon the platinum wires fused into the side of the apparatus, these wires being connected in turn with the induction coil. Under this condition, when both nerves are not stimulated, the amounts of the precipitate are equal in both chambers. However, when one of the nerves is elec-

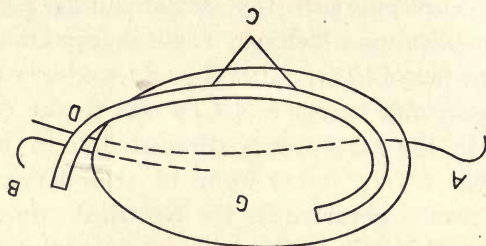


FIGURE 1. Glass weighing plate. A. B. Platinum wire fused in the rear of the glass plate, with hooks. C. The nerve which is stimulated at D. G. The plate proper.

I have the other piece of the same glass out of which this plate is made. This piece of glass is weighed exactly equal to this weighing plate, so that any wet tissue can be weighed very quickly. In order to make results more accurate, no attempt was made to weigh closer than  $\frac{1}{2}$  milligram.

trically stimulated (the distance between the primary and secondary coils was always more than 10 cm. using a red dry battery, the current being barely perceptible on the tongue), not only does the precipitate appear sooner in the chamber in which the excited nerve is placed, but also the quantity of the carbonate is much greater.

To test whether the increase of  $\text{CO}_2$  production from the stimulated nerve is due to the direct decomposing influence of the current, or to the increase of metabolism produced by the passage of a nerve long as we are not concerned with the electrical changes in the nerve, the use of platinum electrodes instead, is not a great objection, provided that the current is weak enough not to decompose the tissue directly, and that the duration of stimulation is not very long.



impulse, the following experiments were performed. If we assume that the condition under which an electrical decomposition takes place is the same both in the living and the dead nerve, then if the increased  $\text{CO}_2$  is due to the current itself, we should expect that when a killed nerve is stimulated by a current, it ought to increase  $\text{CO}_2$  production just as much. When I placed two nerves killed by steam in each chamber, and stimulated only one of them, the stimulated nerve did not give any more  $\text{CO}_2$  than the unstimulated, using the same strength of current employed in the other experiments. In the next place, it was thought that if the increase of  $\text{CO}_2$  is due to direct electrical decomposition, not limited to the point of contact with the electrodes, we ought to get a proportional increase of  $\text{CO}_2$  by altering the distances through which the current directly passes. The fact was, however, that we could produce an increase of  $\text{CO}_2$  production by stimulating with electrodes 2 mm. apart as well as by 15 mm. apart. Increase of  $\text{CO}_2$ , therefore, is due to nervous excitation and not to the direct influence of the electric current itself.

With this consideration, I have proceeded to make a quantitative estimation of  $\text{CO}_2$  from the stimulated nerve in the manner described before. The results are shown in Table V.

**Electrical Stimulation of Medullated Nerve.** — With apparatus 2, the output of  $\text{CO}_2$  from the excited sciatic nerve of the frog has been quantitatively estimated. As shown below, 10 mgs. of the sciatic nerve gives off  $14.2 \times 10^{-7}$  grams of  $\text{CO}_2$  during ten minutes stimulation while the resting nerve of the same animal gave off  $5.5 \times 10^{-7}$  grams for the same units.

**Mechanical Stimulation.** — We have now established the fact that when a nerve is stimulated by an electrical stimulus, it gives off more  $\text{CO}_2$ . In order to prove more conclusively that this  $\text{CO}_2$  production is due to the passage of a nerve impulse, I have employed several other means which are known to have definite influence on excitability of the nerve. So far, the use of these methods has been confined to qualitative experiments, but the results are a sufficient confirmation of the observations made by electrical stimulation. I cite them here as a preliminary report.

Since the ordinary method for mechanical stimulation cannot be applied directly to the nerve in my apparatus in its present form, I used a different method, namely, crushing the nerve. That, when a

TABLE V  
CO<sub>2</sub> PRODUCTION FROM STIMULATED NERVE OF SPIDER CRAB (NON-MEDULLATED)

1	2	3	4	5	6	7	8	9	10
Date	Tempera- ture of room	Weights of nerve in milligrams	Stimulation	Duration of stimulation	c. c. taken from respiratory chamber	Ppt. of BaCO <sub>3</sub> after ten minutes	No. of c.c. of gas which gives ppt. calculated for 10 mg., ten minutes <sup>2</sup>	No. of gas c.c. of gas which does not give ↓, calculated for 20 mg., ten minutes <sup>2</sup>	Original volume of respiratory chamber
Nov. 3	16.8°	20	Stimulated	10 min.	.5	+	1 c.c.	...	9.5
" 3	14	12	"	10 "	.5	+	.6 c.c.	...	"
" 4	14	40	"	10 "	.5	- <sup>1</sup>	...	2.0 <sup>1</sup>	"
" 6	14.8	20	"	10 "	.5	+	1 c.c.	...	"
" 7	16.8	8	"	10 "	.5	+	.4 c.c.	...	"
" "	16.5	8	"	10 "	.5	-	...	.4	"
" 17	16	16	"	10 "	.5	+	.8 c.c.	...	"
" "	17.4	16	"	10 "	.5	+	.8 c.c.	...	"
" "	17.5	8	"	10 "	.5	+	.8 c.c.	...	"
" "	17	8	"	10 "	1.0	-	...	.4	"
" 8	15	11	"	10 "	1.0	+	.8 c.c.	...	"
" "	16	10	"	10 "	1.0	+	1.1 c.c.	...	"
" "						+	1 c.c.	...	"

<sup>1</sup> Killed by steam.

<sup>2</sup> From this we see minimum precipitating volume is .6 c.c., since .4 c.c. is maximum non-precipitating volume:

∴ Total CO<sub>2</sub> output from 10 mg., ten minutes is  
 $1.0 \times 10^{-7} \times \frac{9.5}{.6} \text{ g.} = 16 \times 10^{-7} \text{ g. CO}_2 \text{ at } 14^\circ - 16^\circ$  Compare with Table I



TABLE VI  
CO<sub>2</sub> PRODUCTION FROM STIMULATED SCIATIC NERVE OF FROG (MEDULLATED)

1	2	3	4	5	6	7	8	9	10
Date	Temperature of room	Weight of nerve in milligrams	Stimulation	Duration of stimulation	c.c. taken from respiratory chamber	Precipitation of BaCO <sub>3</sub> after ten minutes	No. of c.c. of gas which gives ↓, calculated for 10 mg., ten minutes <sup>1</sup>	No. of c.c. of gas which does not give ↓, calculated for 10 mg., ten minutes <sup>1</sup>	Original volume of respiratory chamber
March 27	24°	14	Stimulated	10 min.	2	—	...	.8 c.c.	15 c.c.
" "	24	13	"	10 "	1	+	1.3	....	"
" "	24	11.5	"	10 "	1	+	1.15	....	"
" 28	18	13	"	10 "	1.8	+	2.34	....	"
" "	19	8	"	20 "	1	+	1.6	....	"
" "	20	9.5	"	20 "	1	+	1.9	....	"
" "	20	15	"	10 "	1	+	1.5	....	"
" 30	25	14.5	"	10 "	1	+	1.45	....	"
" "	24	9	"	11 "	1	+	...	.99 c.c.	"
" 21	21	9	"	10 "	1.5	—	1.35	....	"
April 3	18	22	"	10 "	.9	+	1.98	....	"
" 4	21	17	"	10 "	1	+	1.7	....	"
" "	22	10.5	"	10 "	1	+	1.05	....	"
" "	25	16.5	"	10 "	1	+	1.65	....	"
" "	23	1.6	"	10 "	1	+	1.6	....	"
" "	21	1.7	"	10 "	1	+	1.7	....	"
" 5	25	9.7	"	10 "	1	—	...	.97 c.c.	"
" "	26	14	"	10 "	1	+	1.4	....	"

<sup>1</sup> Since minimum precipitating volume is 1.05 c.c. and maximum non-precipitating volume is .99, it is obvious that 1.05 c.c. is minimum:  
 $\therefore 1.0 \times 10^{-4} \times \frac{15}{10.5} \text{ g.} = 14.2 \times 10^{-7} \text{ g. CO}_2 \text{ (20° — 22°)}$

protoplasm is smashed, there occurs vigorous chemical changes, is shown by several investigators. Fletcher<sup>28</sup> reports that injured muscle gives off more CO<sub>2</sub> than the normal.

Later he and Hopkins<sup>29</sup> discovered that muscle, under a similar condition, is richer in lactic acid.

Dr. Mathews has observed a similar activity in crushed eggs of *Arbacia*. Quite accidentally, I have discovered that a fresh nerve, too, when crushed with the rough edge of a glass rod gives off more CO<sub>2</sub>. This increase of gas production from the injured nerve, I take to be due to mechanical stimulation. To test this hypothesis, I rendered the nerve unexcitable by means of ether and 0.2 m. solution of KCl, which is known to abolish excitability of a nerve.<sup>30</sup>

Under these conditions, I observed no increase of gas production when the nerve is crushed. Therefore, the metabolism existing in the living nerve must be accelerated by this stimulation when it is injured.

This interpretation, however, is not accordant with that of Fletcher and Hopkins, on muscle. In studies of lactic acid formation in muscle, they found that lactic acid is spontaneously developed, under anaerobic condition, in excised muscle, and that fatigue due to contractions of excised muscle is accompanied by an increase of lactic acid. In an atmosphere of O<sub>2</sub>, there is no survival development of lactic acid for long periods after excision. From a fatigued muscle, placed in O<sub>2</sub>, there is a disappearance of lactic acid already formed. But this disappearance of lactic acid, due to oxygen, does not occur, or is masked, at supraphysiological temperature (e. g., at 30°). Now traumatic injury to an irritable muscle too produces a rapid development of acid. Since, however, in this case the disappearance of lactic acid due to O<sub>2</sub> does not occur, they conclude that one essential condition for this effect of oxygen appears to be the maintenance of the normal architecture of the muscle. Thus they contend that the increase of the lactic acid by mechanical injury is not due to stimulation, but must be due to tissue destruction.

They, however, did not determine, as far as I know, how much the output of CO<sub>2</sub> is affected by treating the injured tissue with O<sub>2</sub>.

<sup>28</sup> FLETCHER: *Journal of physiology*, 1898-9, xxiii, p. 37.

<sup>29</sup> FLETCHER and HOPKINS: *ibid.*, 1906-7, xxxv, pp. 261, 288.

<sup>30</sup> MATHEWS: *This Journal*, 1904, xi, p. 463.



Unless it is proven that  $\text{CO}_2$  production from the injured muscle is quantitatively equivalent to lactic acid formed, their interpretation cannot be applied to the injured nerve, for in the case of the "plateau" of the survival muscle respiration, when in complete loss of irritability, the lactic acid yield remains stationary, Hill calculated that the  $\text{CO}_2$  production corresponds to the amount liberated from the carbonate of the tissue by the lactic acid formed.<sup>31</sup>

Furthermore, if their interpretation is applied to the nerve, the fact that etherized nerves or nerves rendered unexcitable by KCl do not increase  $\text{CO}_2$  output when crushed, cannot be explained. The fact that only excitable nerves when injured increase their  $\text{CO}_2$  production, is a sufficient proof that some sort of stimulation is applied to the nerve when crushed, the tissue destruction, no doubt, following afterward. The increase of  $\text{CO}_2$  production on crushing the living nerve and its absence on crushing the anaesthetized nerve is the point that I want to emphasize here in order to confirm my results obtained by electrical stimulation. I may add here that a perfectly parallel increase of  $\text{CO}_2$  by crushing has been observed in dry seeds, including wheat, wild oats, Lincoln oats, Swedish select oats, leaves of Japanese ivy, and spinal cords of rabbit.<sup>32</sup>

**Chemical Stimulation.** — The study of the nature of chemical stimulation has been so thoroughly made<sup>33</sup> that at first it was thought that chemical reagents would be ideal as stimuli.

It was soon discovered, however, that the presence of minute quantities of a foreign liquid is such a disturbing factor that stimulation by salt solutions could not be used for quantitative experiments. With a qualitative analysis, however, I found a variety of evidences which show that the nerve stimulated chemically gives off more  $\text{CO}_2$ , and that the nerve rendered less excitable by reagents decreases  $\text{CO}_2$  production.

When each sciatic nerve of a frog is isolated and one is left in the normal saline in one case, and in the body of the frog in the other, for the same length of time, and then transferred to the two chambers of the apparatus, if the quantities of the precipitate are compared, it is found that the nerve which has been in normal saline gives more  $\text{CO}_2$ .

<sup>31</sup> HILL: *Journal of physiology*, 1912, xliv, p. 481.

<sup>32</sup> Fuller discussion of these will appear in a subsequent paper.

<sup>33</sup> MATHEWS: *This Journal*, 1904, xl, p. 455; 1905, xiv, p. 203.

It is known that normal saline stimulates frog's sciatic nerves. The different rates at which  $\text{CO}_2$  is produced from the different nerves treated by various concentrations of KCl is equally instructive. It is known that when a nerve is placed in a molecular solution of KCl, a stimulation takes place for a considerable time. Then it finally becomes unexcitable,<sup>34</sup> whereas, .2 m. KCl solution abolishes nervous excitability in a short time without primary stimulation. The  $\text{CO}_2$  production follows exactly analogous to this. The nerve treated with the stronger solution gives more  $\text{CO}_2$  than that of a weaker solution. This was true even after both nerves became unexcitable, showing that the nerve must be giving off more  $\text{CO}_2$  while being stimulated by the stronger solution. Although my quantitative data are not complete at this stage, this preliminary statement is sufficient to show that the nerve chemically stimulated gives off more  $\text{CO}_2$ . It may be added in passing that the different solubility of  $\text{CO}_2$  in the different concentrations of these salts solutions cannot explain these results solely by a physical interpretation, for there is not enough difference in the solubility of  $\text{CO}_2$  in dilute equimolecular solutions of KCl, and NaCl, whose effect on  $\text{CO}_2$  production is so divergent, the former salt diminishing, the latter increasing it.

**Heat Stimulation.** — It may be recalled in Table I that high temperature increases the output of  $\text{CO}_2$  from the resting nerve. A respiratory process should increase proportionally to the temperature. Raising of temperature, however, not only increases the rate of respiration, but also (particularly by sudden changes of it) stimulates the nerve. A very interesting fact is observed in connection with the killing of the nerve. When the nerve is killed gradually by a slow increase of temperature, it gives off more  $\text{CO}_2$  than when killed suddenly, the determination being made after both are killed.  $\text{CO}_2$  production from the dead nerve under this condition must be due to the diffusion of the gas which was formed previously, just as Fletcher's dead muscle is charged with  $\text{CO}_2$  gas. The different outputs of  $\text{CO}_2$  between slowly killed and suddenly killed nerves cannot be accounted for unless we assume that in one case,  $\text{CO}_2$  is produced more while being killed than in the other. Whether such increase of  $\text{CO}_2$  production, however, was due to the acceleration of normal respiration by the slowly increasing temperature, or due to direct stimulation caused

<sup>34</sup> MATHEWS: *loc. cit.*



by heat, or due to both, cannot be decided here unless we consider the relation between excitation and tissue respiration.<sup>35</sup>

It is hoped that we may have a better understanding of this matter when we study the temperature coefficient of normal respiration of the nerve. At present, we are satisfied to state only that there is a strong evidence to support the conclusion that heat, too, increases CO<sub>2</sub> production from the nerve.

### DISCUSSION OF THE RESULTS

**Comparison of Metabolism of Non-Medullated and Medullated Nerve.** — Although it appears ridiculous to attach any significance to the marked similarity in the magnitudes of CO<sub>2</sub> production from non-medullated and medullated nerves, the temptation is irresistible to comment on the high output of CO<sub>2</sub> from the non-medullated nerve fibre. Let us study the Table following (Table VIII), in which a summarized comparison is given.

TABLE VIII

Nerve	CO <sub>2</sub> from resting nerve	CO <sub>2</sub> from stimulated nerve	Rate of increase of CO <sub>2</sub>
Non-medullated (spider crab)	$6.7 \times 10^{-7}$ g. (15° — 16°)	$16. \times 10^{-7}$ g. (14° — 16°)	2.4 times
Medullated (frog)	$5.5 \times 10^{-7}$ g. (19° — 20°)	$14.2 \times 10^{-7}$ g. (20° — 22°)	2.6 “

Since I have found that injury increases the CO<sub>2</sub> production from the nerve, the values I have obtained from cut, or isolated, fresh resting nerves, such as I had to use, may be somewhat greater than the output of normal uninjured nerves would be. But since Alcock<sup>36</sup> has shown that a non-medullated nerve gives a higher electrical response, both in the negative variation and the injury current, the CO<sub>2</sub> increase due to the cut alone will probably be greater in case of the non-medullated nerve than in that of the medullated one. That means that the value of the CO<sub>2</sub> production for the resting uninjured,

<sup>35</sup> See p. 134.

<sup>36</sup> ALCOCK: Proceedings of the Royal Society, 1904, lxxiii, p. 166.

non-medullated nerve should be reduced more from the figures found for the isolated nerve, than that of the medullated one. In other words, by lowering  $6.7 \times 10^{-7}$  gram which is the value for resting, non-medullated, isolated nerves, the rate of increase of  $\text{CO}_2$  by stimulation in the uninjured nerve would become higher than 2.4 times, and probably higher than 2.6 times, which is the rate for the medullated nerve. This greater effect in the non-medullated nerve is what we should expect if our present conception that conduction is in the axis cylinder only, is correct. Before any accurate comparison of the increase of  $\text{CO}_2$  production on stimulation of non-medullated and medullated nerves can be made it will be necessary, however, to determine how much of the  $\text{CO}_2$  from the resting nerve is due to injury alone. Before we consider this point seriously, also, we should determine the metabolic activities of greater numbers of nerves of different animals. Such an investigation is at present useless until we determine more quantitatively the relation between  $\text{CO}_2$  production and the various strengths of stimulation and the degree of excitability. If any uniformity of  $\text{CO}_2$  output in respect to anatomical variations is discovered, light may be thrown on the function of the medullary sheath and other differentiations.

However insignificant these results may be as far as the similar rates of the gas production of these two nerves is concerned, it should be strongly emphasized that technical error plays no part in these determinations. Inasmuch, as we are dealing with such an extremely small amount of the gas, it is quite natural for those who are not familiar with my apparati to suspect, by a hasty inspection of my results, that the small differences I found under different metabolic conditions may be due to mere experimental variations. For this reason, particular attention is called to a detailed description of the quantitative method I used, especially the footnote on page 144, where I have cited a series of determinations of unknown quantities of  $\text{CO}_2$  in testing my apparati. I may repeat here that my experiments with the spider crab and the winter skate were done at Woods Hole<sup>37</sup> during the summer of 1911, while those with the frog were done in Chicago during the winter of 1912. Under these different conditions, I have not only used the different sizes of nerves, but also

<sup>37</sup> I take great pleasure in acknowledging my indebtedness for the kind accommodation offered me by Drs. Lillie and Drew at Woods Hole.



experimented with two different apparati, the respiratory chambers of which have had entirely different capacities.<sup>38</sup>

**Comparison between the Metabolism of Resting Nerves and that of Other Tissues.** — To compare the rate of metabolism of the nerve with that of other tissues is a matter of no great physiological value on account of great variations which do not affect equally the rate of CO<sub>2</sub> production. Simply to give a better picture of the scope of nervous metabolism, however, let us make the following comparison: Since there is no exact determinations made on either the other organs, or the whole animal, in the case of the spider crab, I have quoted those of the nearest crustacea of which data are available. (Table IX).

TABLE IX

Animals	CO <sub>2</sub> per Kg. per hour	Temperature	Determined by <sup>1</sup>
Crustacea (whole animal)			Jolyet and Regnaut
Cray fish ( <i>Astacus</i> ) . . . . .	37.7 c.c.	12°.5	" " "
Crab ( <i>Cancer pagurus</i> ) . . . . .	89.9 c.c.	16	" " "
Lobster ( <i>Homarus vulgaris</i> ) . . . .	54.4 c.c.	15	" " "
Nerve of spider crab ( <i>Labinia caniliculata</i> ) . . . . .	212 c.c.	15° — 16°	Tashiro
Frog:			
( <i>Rana esculenta</i> ) (whole animal) .	.082 gms.	17	Schultz
( <i>Rana temporaria</i> ) (whole animal)	.355 "	19° — 20°	Pott
( <i>Rana pipiens</i> ) (sciatic nerve) .	.33 "	15	Tashiro
( <i>Rana temporaria</i> <sup>2</sup> ) (isolated muscle) . . . . .	.18 "	21	Fletcher
Dog . . . . .	1.325 "	...	Regnaut and Reiset
Man at rest . . . . .	.41 "	...	Pettenkoffer and Voit
" " " . . . . .	.61 "	...	" " "
" " " . . . . .	.37 "	...	Speck

<sup>1</sup> All the figures are quoted from Schäfer's Text Book of Physiology i, pp. 702, 707 and 708, except that of the isolated muscle which I calculated from Fletcher (*loc. cit.*). Fletcher fails to state the weight of a leg, but gives the value .2 c.c. for one-half hour. Hill believes that if we take each leg 6 g. in average, the value will not be far from the truth.

<sup>2</sup> Fletcher fails to state the species of the frog, but it is inferred from Hill's paper.

<sup>38</sup> See the last columns of Table I and Table II.

**Active Nerves.** — That the nerve increases its  $\text{CO}_2$  production approximately 2.5 times when stimulated, is in accordance with our conception of the metabolism of other acting organs. Just how much increase of  $\text{CO}_2$  takes place during functional activity of an organ or organisms depends on conditions as well as on habits of different organs and animals. Pettenkofer and Voit<sup>39</sup> report that a man (weighing 70 kgs.) gives off when working 0.76 grams per kg. per hour, while resting only .56 gram. Barcroft<sup>40</sup> found that the submaxillary gland when stimulated by the chorda tympani gives off 3–7 times more  $\text{CO}_2$  than the resting gland. In the case of contracting muscle, the results are very contradictory. Hermann<sup>41</sup> found that the contracting muscle gave off 9.3 per cent of  $\text{CO}_2$  (by volume) while the resting one, only 1.4 per cent. Tissot<sup>42</sup> and other workers also found a similar increase of  $\text{CO}_2$  from contracting muscle. Minot,<sup>43</sup> working with Ludwig, maintains that there is no relation whatever between  $\text{CO}_2$  production and muscle tetanus. L. Hill<sup>44</sup> and Fletcher<sup>45</sup> both confirmed Minot's work by finding no increase of  $\text{CO}_2$  production from muscular tetanus. According to Fletcher, the increase he found in  $\text{CO}_2$  production from a contracting muscle in a closed vessel is due to the rigor. Under this condition, he believes, increased formation of lactic acid is responsible for liberating  $\text{CO}_2$  already produced. In either case, it is understood that functional activity in the muscle is accompanied by an increase of metabolic activity. It is difficult to compare this increase of metabolic activity of the muscle with that of the nerve unless we determine how much and what kind of metabolism takes place in contracting muscle.

**Respiration Quotient of the Nerve Fibre.** — As quoted before Haberlandt found that a resting nerve consumes 41.7 to 83.4 cmm.  $\text{O}_2$  for 1 gm. for an hour at  $19^\circ - 24^\circ$ . Although he has not determined chemically the production of  $\text{CO}_2$  he could easily read the respiration quotient by means of the index fluid. Thus he found

<sup>39</sup> PETTENKOFER and VOIT: *loc. cit.*

<sup>40</sup> BARCROFT: *Ergebnisse der Physiologie*, 1908, vii, p. 735.

<sup>41</sup> HERMANN: *Stoffwechsel der Muskeln*, Hirschwald, Berlin, 1867.

<sup>42</sup> TISSOT: *Archives de physiologie*, 1894–5, (5) vii, p. 469.

<sup>43</sup> MINOT: *Arbeiten aus der physiologischen Anstalt zu Leipzig*, 1868, p. 1.

<sup>44</sup> L. HILL: See Schäfer's *Text Book of Physiology*, 1898, i, p. 911.

<sup>45</sup> FLETCHER: *Journal of physiology*, 1898–9, xxiii, p. 68.



that the respiratory quotient of the resting and acting nerve is nearly unity. Since he found that  $O_2$  consumption is increased when stimulated, and since the respiration quotient remains constant before and after the stimulation, he concluded that it must give off more  $CO_2$  when stimulated. It is very interesting to compare the  $O_2$  consumption in this experiment with the  $CO_2$  production of mine.<sup>46</sup>

Taking his lowest figure, because he worked in  $19^\circ - 24^\circ$  and I in  $19^\circ - 20^\circ$ , 41.7 cmm. of  $O_2$  amount to .00007 cc. for 10 milligrams for ten minutes. My figure of  $5.5 \times 10^{-7}$  grams for the same units may be translated to .00027 cc. of  $CO_2$  (ignoring temperature and pressure correction). Therefore  $\frac{CO_2}{O_2} = \frac{.00027}{.00007} = 3.8$ , the respiratory quotient.

As I have not determined  $O_2$  consumption of the nerve of *Rana pipiens*, this figure has no particular value, but the fact that the  $CO_2$  production is comparatively higher than  $O_2$  consumption is a matter of considerable interest.

One of the most important observations made by A. V. Hill<sup>47</sup> is the fact that he could not detect any rise of temperature in a frog's nerve as measured by an apparatus which is sensitive to a change of one-millionth of a degree. From this, according to his calculation, he concludes that not more than one single oxygen molecule in every cube of nerve of dimension of  $3.7 \mu$  can be used up by a single propagated nerve impulse. Therefore, he suggested that an impulse is not of irreversible chemical nature but a purely physical change.

Although, I confess, my ignorance makes it impossible to interpret his valuable results from my observations, I may add that these two apparently irreconcilable facts may throw light on the true nature of nervous metabolism. Dr. Mathews has suggested that metabolism in the nerve may be something of the order of alcoholic fermentation, which is not a direct oxidation, and where heat production cannot be so large as  $CO_2$  production, since the energy content of glucose is only a trifle higher than that of the alcohol produced. The comparatively little heat production in the case of working glands is a matter of interest in this connection. At any rate we should not forget the

<sup>46</sup> He used *Rana esculenta*, which, by the way, gives for the whole animal .082 g.  $CO_2$  per kg. per hour at  $17^\circ$  according to Schultz. My frog was *Rana pipiens*.

<sup>47</sup> HILL: Journal of physiology, 1912, xliii, p. 433.

anatomical as well as the chemical differences between muscle and nerve. In this respect the ratio between  $\text{CO}_2$  production and  $\text{O}_2$  consumption from the nerve is suggestive.

The extremely small intake of  $\text{O}_2$  has another point of interest in relation to the general nature of irritability. It has been repeatedly reported that a nerve can remain excitable several hours in an oxygen-free atmosphere, although there is no doubt its excitability diminishes, yet there is a considerable amount of evidence to show that oxygen is very closely associated with the state of excitability. To harmonize these two facts, the oxygen-storage hypothesis has been suggested, by which the exhaustion is attributed to complete consumption of the stored oxygen and that excitability is restored when atmospheric oxygen is readmitted. Without committing ourselves to this hypothesis, I may add that according to Haberlandt's figure, the resting nerve of 10 milligrams will consume only .0042 cc.  $\text{O}_2$  in ten hours. If we take our figure and assume that one volume of oxygen was necessary to produce one volume of  $\text{CO}_2$  (this assumption is made without any significance except to give a liberal estimate), the  $\text{CO}_2$  production would require about .015 cc. of  $\text{O}_2$  for ten hours. And if we assume again that activity will increase  $\text{O}_2$  consumption in proportion of  $\text{CO}_2$  production, then it means that the nerve when stimulated takes up only .03 cc. of  $\text{O}_2$  during ten hours stimulation. I am not aware, at present, of the existence of any method which will surely remove  $\text{O}_2$  as completely as this from a large vessel; and this is a very liberal estimate. My experiences in rendering the air free from  $\text{CO}_2$  encourages me to raise the question, How can one remove every trace of  $\text{O}_2$  from a nerve fibre? Without having a correct criterion for an oxygen-free medium we cannot at present consider definitely any question of the relation of  $\text{O}_2$  to irritability.

#### CONCLUSION

In spite of all the negative evidence against the presence of metabolism in the nerve fibre, we have established three important facts: namely, (1) A resting nerve gives off a definite quantity of carbon dioxide; (2) stimulation increases  $\text{CO}_2$  production; and (3)  $\text{CO}_2$  production from the resting nerve proportionally decreases as irri-



tability diminishes. These facts prove directly that the nerve continuously undergoes chemical changes, and that nervous excitability is directly connected with a chemical phenomenon. There is still another question left, namely, Is there any direct relation between excitability and tissue respiration? To put this question more directly, we may ask: Does excitability depend on the respiratory process in the protoplasm? To answer these questions we must refer to two facts; namely the direct relation between the rate of respiratory activity and the decrease of excitability; secondly, the influence of reagents on CO<sub>2</sub> production and their effects on the state of excitability.

By the studies of CO<sub>2</sub> production by Fletcher<sup>48</sup> lactic acid formation by Fletcher and Hopkins,<sup>49</sup> and heat evolution by A. V. Hill,<sup>50</sup> it has been established that in isolated muscle, respiratory processes decrease when irritability diminishes. In the case of the nerve, as shown in Table 3, CO<sub>2</sub> production reaches this minimum when excitability approaches zero. These relations, however, do not show conclusively that the protoplasmic irritability depends on respiratory activity, for it is quite probable that the dying nerve may alter its physical condition as well, which according to the physical school, may consequently alter the state of excitability.

That irritability is independent of the respiratory processes has been hitherto successfully contended in the case of the dry seed. The works of Horace Brown, Thiselton-Dyer<sup>51</sup> and others indicate that the dry seed can be kept alive at the conditions where no ordinary gaseous exchanges are possible. It is argued, therefore, that life is possible without any metabolic activity.<sup>52</sup> While a definite potentiality for irritability may exist without any metabolic activity, yet that the irritability can persist without respiratory activity, or vice versa, is a matter by no means settled. In the case of ordinary air-dry seed, Waller could demonstrate the response of electrical changes when stimulated although the detection of CO<sub>2</sub> was impossi-

<sup>48</sup> FLETCHER: *loc. cit.*

<sup>49</sup> FLETCHER and HOPKINS: *loc. cit.*

<sup>50</sup> A. V. HILL: *loc. cit.*

<sup>51</sup> THISELTON-DYER: Proceedings of the Royal Society, 1897, lxii, p. 160; *ibid.*, lxy, p. 361.

<sup>52</sup> I am indebted to Professor Crocker for his kind suggestion as to botanical literature.

sible. This failure, however, as he himself expected, was due to the lack of delicacy of the chemical methods for detecting  $\text{CO}_2$ . I observed, with my apparatus that even a single kernel of a dry seed gives off a definite quantity of  $\text{CO}_2$  as long as it is alive. In ordinary condition not only a living dry seed gives off more  $\text{CO}_2$  than the dead one, but also like the nerve, it always gives off more  $\text{CO}_2$  when stimulated by mechanical injury. In the normal condition, therefore, we may safely conclude, there is always metabolic activity as long as the seed is irritable, and that in the different states of irritability, the respiratory activity is proportionately different. At present, therefore, we have no decided evidence which will prevent us from considering excitability as a function of respiration under ordinary conditions. This relation is more directly studied by the use of anaesthetics.

I have already demonstrated that an etherized nerve gives off considerably less  $\text{CO}_2$  than the normal. Such an etherized nerve will not give more  $\text{CO}_2$  when it is crushed. This may be interpreted by some to mean that the etherized nerve may be already dead. This, however, is not the case. This objection, also, I have considered by studying the nerve treated with KCl.

When the nerve is treated with .2 m KCl and then crushed, it does not give an increase of  $\text{CO}_2$  production. Mathews has shown that while a .2 m. KCl solution renders the nerve unexcitable, yet it will recover its excitability by being replaced into  $n/8\text{NaCl}$ . These two facts, therefore, support the idea that any agents that suppress excitability of the nerves also decrease the  $\text{CO}_2$  production and that  $\text{CO}_2$  production by crushing the nerve must be largely due to stimulation. This hypothesis is strikingly supported by similar observations on the dry seed. Etherized seeds give much less  $\text{CO}_2$  and cannot be stimulated to give more  $\text{CO}_2$  by crushing, while under normal conditions, crushing a seed always increases its  $\text{CO}_2$  production. Quantitative experiments in this direction will be given in another paper.

These facts directly support Mathews' hypothesis that substances which suppress irritability must act on the tissue respiration primarily. If such an hypothesis is correct, we can easily picture what is happening in the nerve fibre. Vernon<sup>53</sup> considers that a tissue contains certain substances which can absorb oxygen from their sur-

<sup>53</sup> VERNON: *Journal of physiology*, 1909-10, xxxix, p. 182.



roundings to form an organic peroxide, and by the help of a peroxidase can transfer this to amino acid and carbohydrate molecules bound up in the tissue, just as  $\text{H}_2\text{O}_2$ <sup>54</sup> can oxidize, with the help of an activator, an acid of formula  $\text{R} \cdot \text{CHNH}_2 \text{COOH}$  to  $\text{CO}_2$ ,  $\text{NH}_3$  and an aldehyde  $\text{RCHO}$ , and then oxidize this aldehyde to  $\text{RCOOH}$  and ultimately to  $\text{CO}_2$  and  $\text{H}_2\text{O}$ . Poisons such as  $\text{HNC}$ ,  $\text{NaHSO}_3$  and  $\text{NaF}$ , which he found to decrease  $\text{CO}_2$  production, temporarily paralyzed respiration, he thought, by uniting with aldehyde groups, while formaldehyde, acid and alkali temporarily paralyze  $\text{CO}_2$  forming power of the tissue by destroying the peroxidase. The organic peroxide, though it can still affect some oxidation, cannot of itself carry it to the final  $\text{CO}_2$  stage. Recovery of  $\text{CO}_2$  forming power is due to the regeneration of the peroxidase.

Although I doubt that such a process occurs in nervous respiration, the idea of two similar metabolic phenomena involved in the nervous metabolism is very helpful to understand the behavior of the nerve during continued activity. Most recently Tait discovered that a refractory period has two phases, absolute and relative.<sup>55</sup> When he treated the sciatic nerve of a frog with yohimbine, the relative phase is greatly prolonged, while the absolute one is little affected, a result quite different from other common anaesthetics. Waller<sup>56</sup> has already observed that protoveratrin slows up the positive variation of the nerve, while the negative variation is little influenced. Waller contends that this drug does not alter catabolic change, but retards anabolic activity to a considerable degree. Since pharmacological action on animals of protoveratrin and yohimbine are very similar, Tait concludes that these drugs must attack the nerve in similar manner, and that a refractory period, too, must consist of two phases corresponding to the catabolic and anabolic processes which Waller observed in the case of protoveratrinized nerves. Thus, he considers that his "absolute phase" of the refractory period corresponds to negative variation or catabolic process of the nerve, and the "relative" to the positive return or anabolic. Yohimbine, in other words, retards anabolic processes considerably, thus prolonging the refractory period, or increasing nerve

<sup>54</sup> DAKIN: *Journal of biological chemistry*, 1908, iv, pp. 63, 77, 81, 227.

<sup>55</sup> TAIT: *Journal of physiology*, 1912, xl, p. xxxviii.

<sup>56</sup> WALLER: *Brain*, 1900, xxiii, p. 21.

fatigue easily. These considerations suggest very strongly that the absence of fatigability in the nerve as measured by the ordinary methods, is not a question of absence of metabolism, but merely the speed by which these two processes come to an equilibrium.

Although we have an infinite number of facts still unexplainable, by our present knowledge of nerve physiology, we have established a few new facts around which we may build up some idea concerning this most essential phenomena of living matter, — i.e., irritability. As to the true nature of the nerve impulse, I can only confess my ignorance.

#### SUMMARY

1. All nerve fibres give off  $\text{CO}_2$ . The resting, isolated nerve of the spider crab produces  $6.7 \times 10^{-7}$  gram per 10 milligrams per ten minutes. The frog's sciatic  $5.5 \times 10^{-7}$  grams.

2. When nerves are stimulated they give off more  $\text{CO}_2$ . The nerve of the spider crab claw produces  $16. \times 10^{-7}$  gram when stimulated, the frog nerve  $14.2 \times 10^{-7}$  grams. The rate of increase of  $\text{CO}_2$  by stimulation amounts to about 2.5 times.

3. The  $\text{CO}_2$  output of resting nerve is due to a vital active process.

4. Anaesthetics greatly reduce the carbon dioxide output of nerves and dry seeds.

5. Mechanical, thermal and chemical stimulation also increases the carbon dioxide output of nerves.

6. Single dry living seeds (oat, wheat, etc.) react in most particulars similar to nerves as regards their irritability, relation to anaesthetics, mechanical stimulation and carbon dioxide outputs.

7. The general conclusion is drawn that irritability is directly dependent upon and connected with tissue respiration and is primarily a chemical process. These results strongly support the conception that conduction is of the nature of a propagated chemical change.

To Prof. A. P. Mathews, under whose direction I have carried on these experiments, I express my appreciation and gratitude. For many suggestions, I am under obligation to Dr. F. C. Koch.



## A NEW METHOD AND APPARATUS FOR THE ESTIMATION OF EXCEEDINGLY MINUTE QUANTITIES OF CARBON DIOXIDE<sup>1</sup>

By SHIRO TASHIRO

[From the Department of Biochemistry and Pharmacology, the University of Chicago, and the Marine Biological Laboratory, Woods Hole, Mass.]

IN connection with the study of the metabolism of the nerve fibre, I undertook, at the suggestion of Prof. A. P. Mathews, to work out a method for the detection of exceedingly minute quantities of carbon dioxide. Following a suggestion made by Dr. H. N. McCoy, a very simple method was devised, which I reported first to the Chicago Section of the American Chemical Society;<sup>2</sup> later in conjunction with Dr. McCoy, its further details were reported to the Analytic Section,<sup>3</sup> of the Eighth International Congress of Applied Chemistry. The principle of the new method is as follows:

1. Exceedingly minute quantities of carbon dioxide can be precipitated as barium carbonate on the surface of a small drop of barium hydroxide solution.

2. When a drop of barium hydroxide is exposed to any sample of gas free from carbon dioxide, it remains perfectly clear, but when more than a quite definite minimum amount of carbon dioxide is introduced, a precipitate of carbonate appears, detectable with a lens.

3. By determining, therefore, the minimum volume of any given sample of a gas necessary to give the first visible formation of the precipitate, its carbon dioxide content can be estimated accurately, since this volume must contain just the known detectable amount of carbon dioxide.

<sup>1</sup> One of these apparati was described at the biochemical section, Eighth International Congress of applied chemistry, September, 1912; see also, Journal of biochemistry, 1913, xiv, p. xli.

<sup>2</sup> May 18, 1912.

<sup>3</sup> Original Communication: Eighth International Congress of applied chemistry, 1912, i, p. 361.

I have constructed two apparati, based on this principle, which are especially adapted for the estimation of the output of carbon dioxide for very small biological specimens. With these apparati, one cannot only detect easily a very small amount of gas, given off by a small dry seed, or a small piece of a frog's sciatic nerve, but can also estimate it with considerable accuracy.

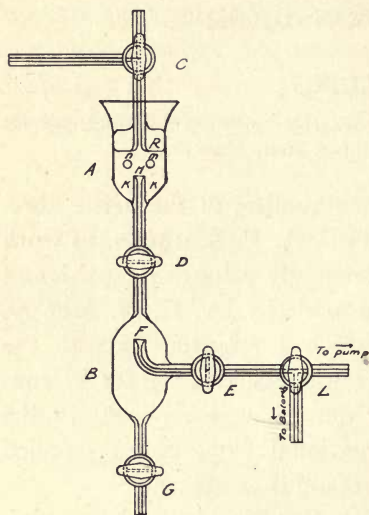


FIGURE 1  
One-third the actual size.

The apparatus shown in Fig. 1 consists of two glass bulbs. The upper bulb A, is a respiratory chamber, having a capacity of about 15 c.c., which can be diminished to 9 c.c. by means of mercury. The lower bulb B is an analytic chamber with a volume of 25 c.c., which can be made to 5 c.c. by filling up with mercury. These two bulbs are connected with a capillary stop-cock D. The respiratory chamber is fitted with a tight glass stopper, R, which is connected to a three-way capillary stop-cock C. This glass stopper is so arranged that the chamber can be sealed by putting mercury above the stopper.

The tubes are thick walled capillaries of about 1 mm. internal diameter, excepting upturned tubes inside the bulbs, which should be rather thin walled, especially at F and H, where it is widened to an internal diameter of about 2 mm. It is important that the glass of which these tubes are made should be of a quality not readily attacked by barium hydroxide.

The details of the method of procedure are as follows:

The apparatus is first cleaned and dried.<sup>4</sup> The specimen is

<sup>4</sup> The apparatus is made in such a way that it can be cleaned and dried in ten minutes without being taken apart. For this, the stop-cock D is closed and E and L are opened. The arm at L is connected to the suction pump. Then a little acidulated water is introduced through G. By closing E, and opening D and G, the excess of water is drained off. Then the process is repeated with distilled water, alcohol, and alcohol ether. The last drying is completed by passing a current of air through G while D is closed.



placed on a glass plate<sup>5</sup> and weighed. The glass plate is hung on n and m, which are electrodes fused into the side of the respiratory chamber A. The chamber is now closed with the stopper R and sealed with mercury. Through L, a connection is made with a pump<sup>6</sup> and about 20 c.c. of mercury is introduced through G. Not too much mercury should be used; its surface should not be within 5 mm. of the cup F. Then wash the whole apparatus with carbon dioxide-free air,<sup>7</sup> which is introduced through C, by successive evacuations. After the evacuation and washing out with pure air, which is repeated three or four times, the pressure inside of the bulbs is made equal to the atmospheric pressure by adjusting it at the nitrometer in the usual fashion. Stop-cock E is then closed, and the space between E and L is evacuated so that the barium hydroxide can rush in, a process which is very advantageous to obtain a clear barium hydroxide solution. Then clear barium hydroxide solution is run in through L. By opening E very slowly and carefully, the solution is now introduced into the chamber so that a small drop stands up upon the upturned end of the capillary at F. Then the connection between the two chambers is closed by D. It is imperative that this drop of the solution should be perfectly clear at the start. If no deposit of barium carbonate forms on the surface of the drop within ten minutes,<sup>8</sup> a portion of the sample gas is drawn into B by withdrawing mercury through G and opening the stop-cock D. The volume of mercury withdrawn, which may be readily determined by volume, or more accurately by weight, gives the volume of the sample

<sup>5</sup> The kind of glass plate used in connection with the nerve and small animals like *Planaria* is shown on p. 120, Fig. 1. (The first paper.)

<sup>6</sup> The pump should be capable of giving a vacuum of at least 25 or 30 mm. of mercury.

<sup>7</sup> Air cannot be freed completely from carbon dioxide by passing it through wash bottles. In my work, carbon dioxide-free air is prepared by shaking air with twenty per cent solution of sodium hydroxide in a tightly-stoppered carboy, fitted with suitable tubes. When this is to be used, it is driven into a nitrometer which is filled with less concentrated alkaline solution (a weak solution is used so that the chamber may not be too dry) by displacing it by running in a solution of sodium hydroxide. After each evacuation, this air is introduced from the nitrometer into the chamber A through stop-cock C.

<sup>8</sup> If no precipitate appears within ten minutes, it is a sure control that the apparatus is free from carbon dioxide.

gas taken from the respiratory chamber, since the pressure in A and B is kept equal to the atmospheric during the transfer.

One now watches the surface of the drop at F with a lens to see whether any formation of barium carbonate occurs within ten minutes. With this apparatus, I have repeatedly introduced accurately known quantities of carbon dioxide of very high dilution into B in the manner just described and as a result have found, with remarkable regularity, that  $1.0 \times 10^{-7}$  gram of carbon dioxide is the minimum amount which will cause a formation of barium carbonate within a period of ten minutes. Smaller amounts of carbon dioxide give no visible results; while larger amounts give a deposit more rapidly, and appear in larger quantities. This minimum detectable amount  $1.0 \times 10^{-7}$  gram is about the amount which is contained in  $\frac{1}{6}$  c.c. of natural air, in which we assume 3.0 parts of carbon dioxide in 10,000 by volume.<sup>9</sup>

In order to determine the concentration of carbon dioxide in the respiratory chamber, one must first find, for the apparatus used, the minimum detectable amount of carbon dioxide. Then one finds, by trial,<sup>10</sup> the minimum volume of gas necessary to give the first visible formation of barium carbonate. This volume must, therefore, contain the known minimum detectable amount of carbon dioxide. From the ratio between this volume and the original volume of the respiratory chamber, out of which this amount is withdrawn, the absolute

<sup>9</sup> LETTS and BLAKE: Proceedings of the Royal Dublin Society, 1899-03, ix, p. 107.

<sup>10</sup> In the case of biological problems, when the specimen gives off carbon dioxide continuously, and sometimes at different rates, varying with the time, it is much simpler not to attempt to determine the minimum volume by a continuous trial with the same sample; but instead to repeat the experiments with a series of samples of known weights for a known time, and determine the minimum volumes which give the precipitates, and the maximum volumes which do not give the precipitates. In this way, it can easily be calculated what is the minimum volume which gives the precipitate for the given weight of the specimen for a given time. Table I on page 114 will illustrate this more clearly.

Another upturned cup H provided in the respiratory chamber A is used in case only the qualitative detection of  $\text{CO}_2$  is wanted. In such a case, the perfectly clear barium hydroxide solution is introduced, after the necessary cleaning and washing, to the respiratory chamber, forming the usual drop at H instead of F. It should be noted that in case a smaller capacity is necessary for the respiratory chamber, the mercury is introduced by a pipette to the bottom of the chamber at K.



quantity of carbon dioxide, given by the specimen, may be computed.

At the suggestion of Dr. F. C. Koch, another apparatus was constructed, which provides a control drop of the barium hydroxide solution, side by side with the other. The apparatus (Biometer) shown in Fig. 2, although it appears complex, is nothing more than apparatus 1, inclined  $90^\circ$ , but each of its chambers is provided with a barium hydroxide cup d and f. It is made of glass consisting of two respiratory chambers, serving also as analytic chambers, connected by a three-way stop-cock L, the other arm of which is connected to one arm of another three-way stop-cock K. Each of the other two arms of stop-cock K is connected to a nitrometer W and X. The nitro-

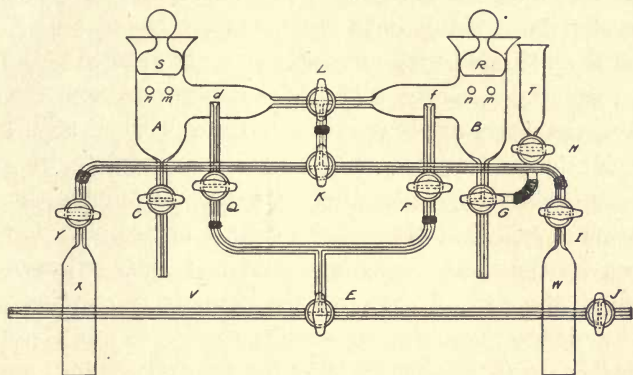


FIGURE 2. Biometer, one-third actual size.

The shaded portions of the apparatus indicate the rubber connection which is first coated by shellac, and then sealed with a special sealing wax. Some parts are also sealed with mercury.

meter on the right, is connected to a carboy with air free of  $\text{CO}_2$ ; and the other, on the left, to a similar reservoir with air free of  $\text{CO}_2$  plus any gas which is desired as a medium for conducting the experiment. Chamber A is drawn to a capillary stop-cock C; chamber B is drawn to the three-way stop-cock G, one arm of which is connected with a mercury burette T, which is used for adjusting the pressure. Both of the chambers have a capacity of 20 to 25 c.c. and are provided with a pair of platinum electrodes n and m, and also with the glass stoppers S and R, which can be sealed as usual with mercury. The pump is connected through J, and the barium

hydroxide solution is introduced through V to d and f, where drops are formed as before.

As stated above, this apparatus can be used for the combined purposes of qualitative detection, quantitative estimation, and comparative determination of the output of  $\text{CO}_2$  from the various biological specimens. It has a decided advantage over the other in the fact that we have a control drop, side by side, under exactly the same conditions, and that the comparative estimation of  $\text{CO}_2$  produced by different specimens can be made very easily and accurately. The detailed method of procedure is described under three different headings:

(a) **For the Qualitative Detection of Carbon Dioxide.** — After the apparatus is cleaned and dried,<sup>11</sup> a weighed tissue is placed on the glass plate and hung on n and m of the chamber A, and no tissue in the other chamber. After both chambers are closed with the stoppers S and R and sealed with mercury, they are so filled with mercury that the remaining volumes in both chambers are now exactly the same. The chambers are now evacuated and washed with pure air. When evacuation and washing with pure air is complete, the pressure is made atmospheric, by adjusting with the nitrometer the connection between A and B is then closed with stopcock L. If any  $\text{CO}_2$  is given off by the tissue, the desposit of carbonate will soon appear on d, while in the control chamber the drop on f remains perfectly clear. In order to avoid any possible error of a technical nature this experiment is repeated by exchanging the chambers, now using chamber B for the respiratory chamber and the other A as a control.

(b) **For Comparative Estimation of  $\text{CO}_2$  from Two Different Samples.** — By repeated quantitative experiments, it was found that the speed with which the first precipitate appears and the sizes of the deposits on the drops at d and f represent corresponding quantities of carbon dioxide. Thus with remarkably simple means, we can determine simultaneously the comparative outputs of the gas from two different tissues or from the same tissues under different conditions. The method of procedure is best illustrated by the following example. Two pieces of the sciatic nerve are isolated from the same frog and exactly weighed. One piece is laid on one glass plate, and the other

<sup>11</sup> This, too, can be cleaned and dried without being taken apart. See footnote on p. 138.



on the other plate in such a way that one part of the nerve lies across the electrodes of the glass plates as shown in Fig. 1, page 120. In this way, when the plates are hung on the electrodes *n* and *m*, any desired nerve can be stimulated with the induction current. These plates are now hung on the electrodes in each chamber, and the usual procedure is followed for the cleaning and the washing of the apparatus to make it  $\text{CO}_2$  free. After the connection between the two chambers is closed by means of stop-cock *L*, the nerve in chamber *A* is stimulated by the current. Then if one can watch over the surfaces of the drops carefully from the start, he finds the first deposit of the carbonate on cup *d* of chamber *A* in which the stimulated nerve is placed. Later, the total amount of the precipitates grows much larger in the case of this cup. This increased output of the carbon dioxide from the stimulated nerve, thus observed, can be duplicated by repeating the similar experiment, after exchanging the chambers, as usual. This comparative estimation can be more accurately made by exact quantitative measurement, the method for which the following will illustrate.

(c) **For Quantitative Measurement of Gas.**—The detailed method is exactly analogous to that of apparatus 1. Here we use chamber *B* as the respiratory chamber and *A* as the analytic chamber. Barium hydroxide should be introduced into chamber *A* only at *d*, and the stop-cock *F* is always closed except at the time of washing. The pressure should be adjusted by mercury burette *T*, or by the potash bulb of the nitrometer. In case the mercury burette is used, the remaining volume in the respiratory chamber should be recorded.<sup>12</sup> The introduction of a known amount of gas from the respiratory chamber *B* to the analytic chamber *A* is accomplished by withdrawing the mercury from *C* into a very narrow graduated cylinder, while the stop-cocks *L* *G* and *H* are opened. After a quick adjustment of the mercury burette to equalize the pressure, the stop-cock *L* is closed and the presence of carbonate is looked for exactly in the same manner as described in connection with the other apparatus, determining the minimum volume that gives the precipitate for the known mass of tissue for a known time.

<sup>12</sup> The bulbs are marked at the point where their capacity became 15 c. c. by introducing mercury. The variation of capacity can easily be read by noting the mercury burette.

In summarizing, I may emphasize the following points:

1. Particular care must be taken to test the air-tightness of the apparatus.
2. Purifying the air must be done with greatest care, as this is essential.
3. The apparatus must be perfectly dry.
4. A weak suction pump cannot be compensated by frequency of washing.
5. As long as the ratio between the c.c. taken from the chamber and the original volume of the chamber is needed, it is most important to have the pressure in A and B equal to the atmospheric. If this is accomplished we can neglect any caution against pressure and temperature variations — a correction which is always necessary for ordinary methods of analysis of exceedingly minute quantities of any gas.

In devising this method and in constructing this apparatus, I am under great obligation to Professors McCoy and A. P. Mathews and to Dr. F. C. Koch.

In order to test the accuracy with which an estimate of concentration of carbon dioxide could be made, many determinations were carried out with samples of air which contained accurately known concentrations of carbon dioxide prepared by Dr. F. C. Koch. The experimenter did not learn the concentrations of the samples until after the analysis had been completed. In making up the test samples, pure carbon dioxide, made by heating sodium bicarbonate was diluted with the carbon dioxide free air several times in succession, as illustrated by the following example: 5.5 c.c. of pure carbon dioxide was diluted to 52.0 c.c. over mercury and thoroughly mixed; 5.5 c.c. of the first mixture was diluted to 52.0 c.c.; 1.1 c.c. of the second was diluted to 50.7 c.c.; of this third mixture 5.6 c.c. was received from Dr. Koch. I diluted this a fourth time to 255.6 c.c. to form a mixture to be analyzed. The following observations were made: 0.5 c.c. was introduced into the apparatus and produced no precipitate in ten minutes; 0.5 c.c. more of the same sample, gave no precipitation in another interval of ten minutes; 0.5 c.c. more, a total of 1.5 c.c., was run into the bulb. In six minutes the first evidence of a precipitate appeared on the surface of the drop at d of apparatus 2 and in eight minutes was well developed. Since



the amount of carbon dioxide required to give the precipitate is  $1.0 \times 10^{-7}$  grams, this amount is contained in 1.5 c.c. of the sample or 1 c.c. contained  $6.7 \times 10^{-8}$  grams of carbon dioxide. The amount of carbon dioxide actually contained in the sample was

$$\frac{5.5 \times 5.5 \times 7.1 \times 5.6}{52 \times 52 \times 50.7 \times 255.6} \text{ c.c.} = 6.2 \times 10^{-8} \text{ grams.}$$

In six such determinations, all made with samples the concentration of which were unknown to the experimenter at the time of the analysis, the results given in the following table were obtained:

Volume of sample required to give a precipitate	Weight of carbon dioxide in one c.c.	
	Found	Taken
1.0 c.c.	$1.0 \times 10^{-7}$ g.	$0.92 \times 10^{-7}$ g.
.5 c.c.	$2. \times 10^{-7}$ g.	$2.3 \times 10^{-7}$ g.
.55 c.c.	$1.82 \times 10^{-7}$ g.	$1.83 \times 10^{-7}$ g.
1.5 c.c.	$.67 \times 10^{-7}$ g.	$0.62 \times 10^{-7}$ g.
2.25 c.c.	$.45 \times 10^{-7}$ g.	$0.45 \times 10^{-7}$ g.

The first of these is the fact that the  
 second of these is the fact that the  
 third of these is the fact that the  
 fourth of these is the fact that the  
 fifth of these is the fact that the  
 sixth of these is the fact that the  
 seventh of these is the fact that the  
 eighth of these is the fact that the  
 ninth of these is the fact that the  
 tenth of these is the fact that the

Table 1		
Year	Population	Area
1900	1,000,000	100,000
1910	1,200,000	120,000
1920	1,400,000	140,000
1930	1,600,000	160,000
1940	1,800,000	180,000
1950	2,000,000	200,000



## ERRATA

IN JUNE NUMBER OF THE AMERICAN JOURNAL OF PHYSIOLOGY  
(Vol. XXXII, No. II)

Substitute "apparatus" for "apparati" in the following places:

Page 110, lines 7, 11, 23.

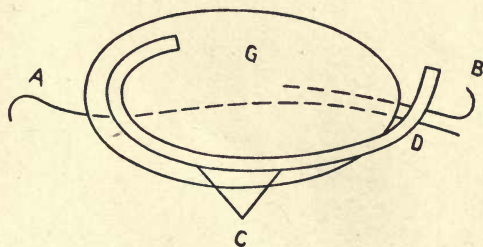
Page 129, line 1.

Page 137, line 28.

Page 144, line 16.

Substitute "7.1 cc." for "1.1 cc." on page 144, line 29.

In figure 1, page 120, correct as indicated in the following drawing









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